The Exposome Project for Health and Occupational Research

Protocol of study on working life exposome and respiratory health

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1. Background

Chronic obstructive pulmonary disease (COPD) and asthma are common chronic diseases. According to the Global Burden of Disease-project for 2019, COPD resulted in 74.4 million Disability-Adjusted Life Years (DALYs) in 2019, representing 71.9% of total chronic respiratory disease DALYs. The corresponding numbers for asthma were 21.6 million DALYs representing 20.8% of total DALYs from chronic respiratory diseases (GBD 2019, de Marco *et al.* 2004). Occupational and non-occupational exposures both contribute substantially to the burden of asthma and COPD (Driscoll *et al*. 2005). The evidence originates from data based on the 'one exposure, one disease' principle (Lytras *et al.* 2019), which severely hampers a valid estimation of the true importance of the totality of occupational and related non-occupational exposures for health. When assessing associations between exposure and respiratory symptoms or disease considering the total burden of exposures is crucial, since most individuals are daily exposed to a multitude of both potentially hazardous and beneficial exposures. The exposome has been defined as "an integrated function of exposure on our body including what we eat and do, our experiences, and where we live and work" (Vermeulen *et al.* 2020). The working life exposome is an important and integral part of the exposome concept (Pronk *et al.* 2021, *under review*).

An understanding of the key biological pathways between occupational exposures and lung disease and respiratory symptoms is limited. Biomarkers are available for short-term non-occupational effects of exposure to e.g. ozone, but valid biomarkers for occupational exposures that is relevant and predictive for the development of occupational respiratory diseases are lacking. A few biomarkers with a clear relation to COPD and reduced lung function have been identified, including the proteins YKL-40 (Chitinase-3-like protein 1) and CC-16 (Club/Clara cell secretory protein). **YKL-40** plays a major role in tissue injury, inflammation, tissue repair, and remodeling responses in diseases characterized by inflammation and tissue remodeling (Zhao et al. 2020). Elevated YKL-40 in serum has been reported to be correlated with a lower lung function (Chupp *et al.* 2007;Guerra *et al.* 2013) and accelerated decline in lung function (Guerra *et al.* 2013). Serum YKL-40 were also elevated in patients with asthma (Chupp *et al.* 2007;James *et al.* 2016) and COPD (James *et al.* 2016) compared with healthy control subjects, and correlated positively with the severity of asthma (Chupp *et al.* 2007). However, YKL-40 levels has also been reported to be strongly and significantly correlated with age (Guerra *et al*. 2013;Johansen et al. 2008;Schultz & Johansen, 2010) and tobacco pack-years (Guerra *et al.* 2013), but stable and with no diurnal variation in healthy subjects (Johansen *et al*. 2008). **CC-16** is suggested to be a protective and anti-inflammatory mediator in the airway inflammatory process (Broeckaert & Bernhard, 2000;Lakind *et al.* 2007). In population-studies level of serum CC-16 has been reported to be inversely associated with accelerated decline of FEV_1 in asthmatics (Rava *et al.* 2013), but also with an increased risk of developing stage 2 airflow limitation (Guerra *et al*. 2015). Also in clinical studies a decreased circulating level of CC16 have been reported in asthmatics (Shijubo *et al.* 1999) and a faster subsequent decline of FEV_1 among patients with COPD (Vestbo et al. 2011;Park *et al.* 2013). The serum CC-16 level is reported to transiently increase following acute exposure to pulmonary irritants (e.g. cigarette smoke, fire smoke, bitumen fume, bioaerosols, chlorine, ozone) (Bernard *et al.* 1997; Stockfelt *et al.* 2012;Ulvestad *et al.* 2007;Steiner *et al*. 2005;Rava *et al.* 2013) and it decreases after chronic exposures to cigarette smoke, occupational exposure to silica and firefighting (Bernard *et al*. 1992;1994;1997;Beci *et al*. 2021). Serum CC-16 increases with age presumably due to age-related decrease of the GFR, while most reports on

circadian variations (Helleday *et al*. 2006;Stockfelt *et al*. 2012). The impact of occupational exposures on both YKL-40 and CC-16 and subsequently lung disease risk is unknown (Guerra *et al.* 2013, 2015).

There are also several short-lived biomarkers of inflammation that are used in assessment of the acute phase reaction related to inflammation or tissue damage triggered by chemical exposures or immunological and allergic reactions. Increased **c-reactive protein (CRP)** is the principal downstream mediator of the acute-phase response following an inflammatory event, and has been reported to be associated with poor prognosis of COPD and airway inflammation (Lock-Johansson *et al*. 2014;Mendy *et al.* 2018). The interleukins (IL-1β, 2, 4, 5, 6, 8, and 10) and TNF-α are cytokines that play a central role in the inflammatory process (Bertsch *et al.* 2015;Fajgenbaum and June, 2020). The level of these immune markers has been reported to be increased after acute exposure to both chemical and particulates (Prunicki *et al.* 2020), and may serve as surrogate markers of airway inflammation in asthma. The inflammation-induced release of pro-inflammatory cytokines through mechanisms in which **epigenetic alterations** are known to play important roles in the specificity and duration of gene transcription. However, little is known about the temporal or aberrant DNA methylation patterns in healthy or asthmatic subjects in response to short term exposure to low levels of irritants and/or allergens. Finally, also exhaled breath (EB), exhaled breath condensate (EBC) and exhaled breath aerosol (EBA) are matrices containing biomarkers of exposure and effect that may represent promising non-invasive biomarkers of respiratory diseases.

For implementation of preventive measures at the workplace, it is important to not only identify the specific agents or exposure, but also to know the exposure metric of interest, i.e. the roles of peak-, short- and long-term exposures in relation to lung function and disease. Also, insight into impacts on vulnerable subgroups is critical to tailor preventive measures. There are some indications of gender differences in age-related effects on lung function and effects of occupational exposures on lung disease (Dimich-Ward *et al.* 2012), and that impact off occupational exposures could be modified by developmental disadvantage in early life. Still, the results are inconclusive.

Knowledge of the exposome involving both occupational and non-occupational risk factors (i.e. general environment, lifestyle, behavioural and socio-economic) and genetic make-up, as well as the need for better insights in relevant exposure metrics and biological pathways, is imperative for disentangling the complex relationship between environment and respiratory diseases. In the present project, WP6 on working-life exposome, lung function, and obstructive lung diseases among men and women, we aim at obtaining better and more complete knowledge by combining two study approaches investigating how both short- and long-term working-life exposures are related to respiratory health. The evidence on working-life risk factors of respiratory health gained in the EPHOR mega cohort will be complemented with state-of-the-art methods for new data collection on the external and internal exposome. This will advance knowledge on how long- and short-term occupational and non-occupational exposures interact with individual biological pathways in relation to respiratory disease risk. Furthermore, we will investigate potential gender differences. The focus on respiratory disease, a major working-life related health outcome, is particularly important in view of the potential for a prolonged productive working-life.

2. Aim, hypotheses and research questions

Overall aim of WP6

The overall aim of WP6 is to evaluate the impact of the working-life exposome on respiratory health. The specific objectives are:

- To apply new targeted and agnostic exposome methods to collect individual level data on the external and internal exposome in selected existing cohorts with extensive respiratory outcome data
- To examine how the long-term and short-term external working-life exposome affect lung function, lung function decline, and risk of asthma and COPD, and investigate if this is influenced by vulnerability factors such as gender and age
- To identify key biological pathways and markers for internal exposure and respiratory health effects associated with the external working-life exposome, using biomonitoring, targeted biomarker assays and agnostic genetics, epigenetics, transcriptomics and proteomics

Key research questions

- How do long-term and short-term working-life exposome affect lung function, and risk of asthma and COPD?
- Are these associations influenced by vulnerability factors such as gender and age?
- What are the key biological markers for exposure and respiratory health?
- What are the key biological pathways linking exposome exposure to respiratory health?

Long-term exposure and respiratory health

Key hypothesis

Long-term lung function decline, asthma and COPD status are related to biological markers of susceptibility, and these associations are modified by long-term combined occupational exposure (**Figure 1**).

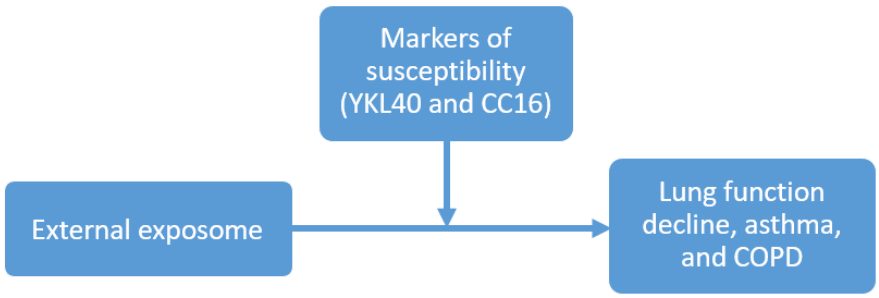

***Figure 1**. Hypothesis for long-term exposure and respiratory health.*

Research question 1:

a) How do long-term lung function decline and new-onset COPD relate to cumulative combined external occupational exposure assessed from job titles and EuroJEM?

b) Do age at exposure, sex, or biological markers of susceptibility (YKL40 and CC16) modify the association between cumulative combined external exposure and long-term lung function decline and new-onset COPD?

Research question 2:

a) How does new-onset asthma relate to recent and cumulative combined external exposure assessed from job titles and EuroJEM?

b) Do age at exposure, sex, or biological markers of susceptibility (YKL40 and CC16) modify the association between recent and cumulative combined external exposure and new-onset asthma?

Research question 3:

a) How does cumulative combined external exposure assessed from job titles and EuroJEM affect disease severity and prognosis among persons with asthma or COPD at baseline?

Short-term exposure and respiratory health

Key hypothesis

Short-term effect biomarkers and changes in respiratory symptoms and lung function are affected by short-term occupational exposure, and these associations are modified by age, sex and biological markers of susceptibility (**Figure 2**).

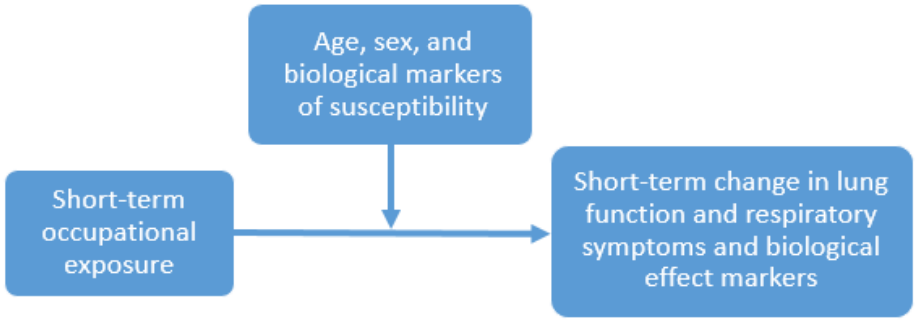

***Figure 2**. Hypothesis for short-term exposure and respiratory health.*

Research question 1:

a) How do short-term effect biomarkers relate to short-term (weekly) occupational exposure (measured by biomarkers and in the environment) among persons with mild asthma?

b) Do age, sex, or long-term biological markers of susceptibility modify the association between short-term occupational exposure and short-term effect biomarkers?

Research question 2:

a) How does cross week and daily changes in lung function and respiratory symptoms relate to cross week and daily (changes in) external occupational exposure among persons with mild asthma?
a) Do age, sex, or biological markers of susceptibility modify the association between change in external occupational exposure and changes in lung function and respiratory symptoms?

3. Material and methods

3.1 Population and design

The study population is based on two population-based cohorts with abundant information on health and risk factors including lung function, respiratory symptoms, asthma, COPD, anthropometric measures and life-long job histories with a planned follow-up in 2021; the European Community Respiratory Health Survey (ECRHS) (Burney *et al.* 1994) and the French Constances cohort (Goldberg *et al*. 2017; Henny *et al.* 2020). Both cohorts have a biobank. From the ECHRS cohort, 11 study centers from 6 European countries (Spain, Sweden, Norway, Estonia, Iceland and Denmark) and Australia will be included (**Table 1**). From the Constances cohort in France, as participants were included in 24 different centers from different regions, a different selection process will be used: participants will be selected according to eligibility criteria (see below) and invited to take part of the study.

Table 1. *Overview of the participating countries and study centers.*

Country	Study center
Australia	Melbourne
Denmark	Aarhus
Estonia	Tartu
France	Constances (Whole country)
Iceland	Reykjavik
Norway	Bergen
Spain	Barcelona Galdakao (ony long-term study) Huelva (only long-term study) Albacete (only long-term study)
Sweden	Göteborg Umeå Uppsala

In EPHOR, 2500 participants from ECRHSIII (2010-12) and 1,500 participants from Constances (2018-2020) will be invited (**Figure 3**). Based on previous follow-ups in these cohorts, a participation rate of 75% is expected, resulting in 3000 individuals with follow-up data (long-term study). For a sub-group, a cross-week panel study among persons with mild asthma and/or allergic rhinitis, and occupational exposure to asthmagens will be conducted (short-term study).

Eligible participants and invitation to participation

Long-term study

For the **ECRHS cohort**, we will invite by mail all participants, who participated in the clinical examinations in ECRHSIII. In the invitation, we plan to mention that we will call the participants to schedule the physical examination.

For the **Constances cohort**, we will include participants matching the following criteria:

- Recruited in 2018-2020 (baseline for the Constances cohort biobank)
- With at least one valid lung function measurement (attended lung function testing, have good reproducibility, have good acceptability)
- Have ever worked
- With data on occupational history (with job titles / job codes)
- With data on ever asthma or asthma symptom score
- Have blood samples in the biobank
- Data on main potential confounders: age, sex, height, weight, smoking

Selected participants will be invited for one additional lung function test. The possibility to include preferably participants in centers where the same device can be used for the second lung function measurement as for the first one performed at baseline will be examined.

Short-term study

Around 700 subjects will be invited to participate in the short-term study. In the invitation for the long-term study, we will inform the participants that some participants will be invited to participate in additional examinations, including biological measurements (spirometry, blood samples, urine samples, exhaled breath), daily app questionnaires, and wearing four sensor systems (i.e. the cross week study, see below) (**Figure 3** and **Figure 4**).

For the **ECRHS cohort**, at the visit to the study clinic for the physical examination for the long-term study, we will determine each participants' final eligibility for the short-term study by going through a list of questions related to inclusion and exclusion criteria for the short-term study. The inclusion criterias for participants eligible for the short-term study are based on: 1) participation in the long-term study (preferably for ECRHS, not for Constances), 2) mild asthma and/or allergic rhinitis, and 3) occupational exposure to one or more asthmagens at any level (all agents listed in the occupational asthma specific JEM by Le Moual *et a*l (2018; Asthma-specific Job Exposure Matrix (inserm.fr)). Definition of mild asthma and or allergic rhinitis is responding "yes" to one or more of the following: Ever asthma (doctor-diagnosed), current asthma, current use of asthma medication, one or more asthma symptoms (Sunyer *et al*. 2007), ever wheeze from questions on animal exposure, dust or pollen, or allergic rhinitis. In ECRHS III, 493 participants fulfill the criteria for inclusion into the short-term study.

For **Constances**, the participants will be selected among those: (1) currently holding a job exposed to one or more asthmagens to any level according to the OAs JEM Asthma-specific Job Exposure Matrix (inserm.fr) and Le Moual *et al.* (2018) [data not available currently, may be available mid-

2021], and (2) with mild asthma and/or allergic rhinitis, defined by a positive answer to one or more of the following: ever asthma (doctor-diagnosed); current asthma; current use of asthma medication; one or more asthma symptoms (Sunyer et al. 2007); ever wheeze ("have you ever had attacks of breathlessness at rest with wheezing in the chest?"; allergic rhinitis ("have you ever had nasal allergies, including hay fever"); currently aged <60 years to minimize the risk they will be retired.

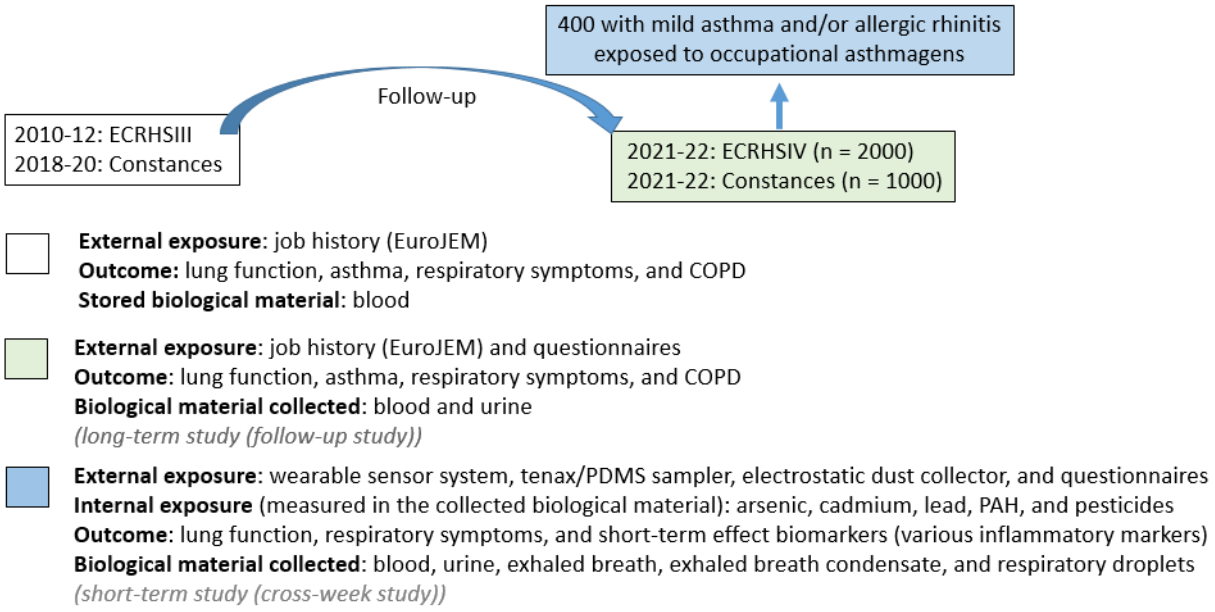

***Figure 3**. Study population and design of the long- and short-term studies.*

3.2 Collection of data

A. Long-term study

In the long-term study, baseline is 2010-2012 in the ECHRS cohort (ECRHS III) and 2018-2020 in the Constances cohort.

A.1 Exposure measurements

ECRHS

Occupational history will include information from the following sources:

- Lifelong job history based on interview data about job titles (source: ECRHSIII (question 37) and ECRHSIV (question 33) main interview questionnaire, **Appendix A**)
- EuroJEMs based on job titles and existing exposure data through the development of harmonised and enhanced job-exposure matrices (JEMs) (from WP2, to be completed)
    - Chemical
    - Physical
    - Ergonomic

    - Work organizational
    - Psychosocial
    - Lifestyle and other non-occupational aspects
- Chemical exposures, e.g. cleaning agents at work (source: ECRHSIII/IV main interview questionnaire, **Appendix A**, and ECRHSII questionnaire on occupational cleaning and disinfecting agents)
- Sun exposure at work (source: ECRHSIII/IV questionnaire, **Appendix B**)

Constances

- Lifelong job history based on the Job History Questionnaire at baseline (currently coded with French nomenclatures; crosswalk with ISCO-ISIC in progress, **Appendix I**)
- EuroJEMs (from WP2, to be completed, see above)
    - Chemical
    - Physical
    - Ergonomic
    - Work organizational
    - Psychosocial
    - Lifestyle and other non-occupational aspects
- Chemical exposures e.g. cleaning agents (source: baseline questionnaire on occupational exposures)
- Sun exposure – occupational exposure (baseline questionnaire on occupational exposures)
- Working time (source: baseline questionnaire on occupational exposures)

A.2 Health outcomes

The outcomes of the long-term study focus on chronic respiratory effects and will be assessed based on information from questionnaires and test of lung function:

- Change in lung function between baseline and follow-up, measured as pre-bronchodilator FEV_1 and FVC in ECRHS (sources: ECRHSIII/IV, **Appendix C**) and Constance (Constances database). In ECRHS change in post-bronchodilator FEV_1 and FVC will also be assessed.
- Incidence of asthma, only new cases during follow-up. Defined as ever asthma (yes, no) based on interview information on ever asthma (sources: ECRHSIII/IV main interview questionnaires, **Appendix A**, question 15, 15.1 and 15.11; Constances Follow-Up (FU) questionnaires, administrative data)
- Prevalence of current asthma, status at baseline and at follow-up. Defined as current asthma (yes, no) based on report of asthma attack during the past 12 months and/or current use of asthma medication (sources: ECRHSIII/IV main interview questionnaire, **Appendix A**, questions 15.6 and 15.11; Constances baseline and FU questionnaires, administrative data)
- **Change** in asthma symptom score between baseline and follow-up. The score is based on the number of five asthma symptoms and ranges from -5 to 5 (Sunyer et al. 2007). It will be

investigated both as a continuous variable and a categorical variable (source: ECRHSIII/IV main interview questionnaire, **Appendix A**, questions 1-5; Constances FU questionnaires)

- Asthma severity and asthma control: The multidimensional scales following Global Initiative for Asthma (GINA) guidelines (Bateman et al. 2008) will be used to define asthma control (controlled asthma, partly controlled asthma, uncontrolled asthma) as described in previous studies on ECRHS (Cazzoletti et al. 2007;Le Moual et al. 2014), and will be a combination of diurnal and nocturnal respiratory symptoms, asthma attacks, activity limitations, lung function and exacerbations.
- Incidence of COPD during follow-up. Status at baseline and at follow-up. COPD is defined as an FEV_1/FVC ratio below the lower limit of normal (the lower 5^{th} percentile) as measured by spirometry (Swanney et al. 2008) and/or according to interview information about doctor diagnosed COPD (sources: ECRHSIII/IV, main interview questionnaire **Appendix A**, question 17; Constances FU questionnaires, administrative data).

A.3 Potential confounders

Covariates will be defined *a priori* based on the existing literature and further evaluated in causal diagrams using directed acyclic graphs (DAGs). At a minimum, the following variables will be considered:

- Age (source: ECRHSIII and Constances)
- Sex (source: ECRHSIII and Constances)
- Height (source: ECRHSIII/IV, **Appendix D;** Constances, clinical SOP)
- Weight (source: ECRHSIII/IV, **Appendix D;** Constances, clinical SOP)
- Waist circumference (source: ECRHSIII/IV, **Appendix D;** Constances, clinical SOP)
- Hip circumference (source: ECRHSIII/IV, **Appendix D;** Constances, clinical SOP)
- BMI will be calculated from weight and height, kg/m^2
- Bioimpedance (source: ECRHSIII/IV, Appendix D;Constances do not have these data)
- Diet, measured by a 239-item food frequency questionnnaire (source: ECRHSIII/IV, **Appendix E**;Constances, **Appendix III**)
- Chemical exposures e.g. cleaning agents at home (source: ECRHSIII/IV main interview questionnaire on cleaning agents at home, **Appendix A**)
- Sun exposure: questionnaire on lifelong exposure outside work (source: ECRHSIII/IV questionnaire on sun exposure, **Appendix B**; Constances baseline questionnaire)
- Physical exposures, e.g. physical activity (both occupational and spare time) (source: ECRHSIII/IV questionnnaire, **Appendix F**; Constances, **Appendix II**).
- Smoking (source: ECRHSIII/IV main interview questionnaire, **Appendix A**; Constances, **Appendix XIII**)
- Alcohol consumption (source: ECRHSIII/IV, **Appendix A** and Food Freqency Questionnaire, **Appendix E;** Constances, **Appendix XV**)
- Socioeconomic status, determined by level of education.

- ECRHS main interview questionnaire ECRHS IV (**Appendix A**):

  What is your highest educational level?

  a) Up to the minimum school leaving age
  b) Secondary school/technical school past the minimum age
  c) College or University
- Constances questionnaire have several measures of education which will be aggregated into the same categories as ECRHS (source: **Appendix I**).

- Sleep quality (source: ECRHSIII/IV, **Appendix G**; Constances, **Appendix IX**)
- Atopic disposition (family history of atopic disease) (source: ECHRSIII/IV main interview questionnnaire, **Appendix A**; Constances, proxy current allergic rhinitis).
- Atopic status, determined by elevated specifc IgE antibodies in serum, by skin prick testing, or by interview information on hay fever (source: ECRHSIII/IV main interview questionnnaire, **Appendix A**; Constances, information on hay fewer at baseline).

A.4 Potential effect modifiers

Based on the exitsting literature, the following potential effect modifiers have been selected:

- Age (source: ECRHSIII and Constances)
- Sex (source: ECRHSIII and Constances)
- YKL40 and CC16 (biological markers of susceptibility to respiratory disease) will be measured in order to investigate how occupational exposures modify the associations between YKL40 and CC16 and long term respiratory health:
  - YKL40 will be measured in biobanked serum (all, approx. 2,500 samples) at baseline (ECRHSIII and Constances I) and for a sub-sample (n=400) also at follow-up
  - CC16 will be measured in biobanked serum (all, approx. 2,500 samples) at baseline (ECRHSIII and Constances I) and for a sub-sample (n=400) also at follow-up
- Atopic disposition (family history of atopic disease) (source: ECRHSIII/IV main interview questionnnaire, **Appendix A**)
- Atopic status, determined by elevated specifc IgE antibodies in serum by skin prick testing, or by interview information on hay fever (source: ECRHSIII/IV main interview questionnnaire, **Appendix A**; Constances information on hay fever at baseline)

B. Short-term study

A sub-sample of participants (n=400) from the long-term study with mild asthma and/or allergic rhinitis **and** occupational exposure to airbourne allergens and/or irritants will be selected based on their job title and replies to questions on asthma status and symptoms and other questions asked in the long-term study indicating respiratory susceptibility. The short-term study is a cross week study (one work week) and aims to investigate the association between short-term internal and external occupational exposures and changes in lung function, respiratory symptoms and effect biomarkers relevant for the respiratory diseases of interest (figure 4). At the beginning and end of the week, the particpants from ECRHS will visit a study clinic, while participants from Constances will be visited by a nurse at home. A lung test will be performed and urine and blood samples,

exhaled breath (EB), exhaled breath condensate (EBC) and respiratory droplets (RD) will be collected. During the week, the participants will wear a sensor system with multiple devices (developed by WP1) and an electrostatic dust fall collector (EDC) will be placed in their home (see below). The participants environmental exposures to chemicals and particles during their day-to-day working life, which contribute to the person's working-life exposome, will be measured. In addition, the participants will perform lung function tests on portable, hand-held spirometers three times daily, and answer daily app questions about respiratory symptoms, work time, use of personal protective equipment (PPE), commuting, physical activity, sleep quality and wellbeing. There is evidence for circadian variations in several of the biomarkers being investigated, including CC-16, lymphocytes and DNA methylation content. Hence, all biological samples will be sampled in the morning, hence reducing this source of variation.

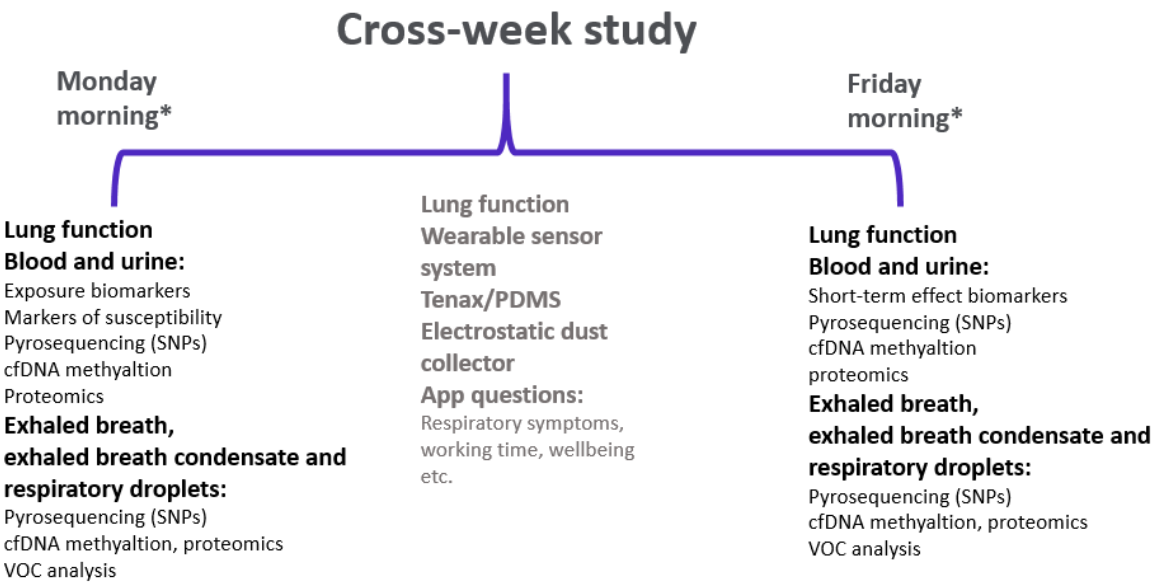

***Figure 4**. Details on the cross week study. *The time for the measurements may be adjusted according to individual working hours.*

B.1 External exposures

- Job title (source: ECRHSIII main interview questionnaire, **Appendix A**, question 37)
- Screening questionnaire during the clinical examination with information on daily pollen count (if available) and when they had their last work shift (source: **Appendix Q**).
- Working time (source: "How am I" App from WP1, **Appendix R**)
- Ambient air pollution: address (ZIP code) of current residence and current workplace, as well as commuting to and from work, will be recorded and geocoded for environmental exposure assessment (source: ECRHSIII/IV main interview questionnaire, **Appendix A** and Screening questionnaire, **Appendix Q**). For Constances, current address is already geocoded. In respect to communting the following question is asked: "What means of transport do you most ofen use for commuting to work (personal vehicle, public transport, other)?

- Chemical exposure (environmental) during participants day-to day working life will be measured using a Tenax/PDMS sampler (combined Tenax TA tube and PDMS (silicone) tubing) (source: WP1, **Appendix S**). The passive sampler are specially designed and consist of a small aluminium tube filled with an adsorbent (Tenax TA) to capture VOC. A piece of silicone hose is attached around the tube (PDMS), which captures SVOCs. Around the silicone hose there is protective mesh cover to avoid contact with clothes and skin (hands). The sampler will be worn at the chest (close to the breathing zone) a full work week both at work and outside work where possible (**Figure 5**). The sampler and analytes are given below. A total of 400 samples will be collected, one per participant. For samples collected on PDMS, **number in brackets** are number of analysis from the 400 samples, remaining 350 samples will be stored in the freezer. The following analytes will be analyzed:
    - Semi-volatile organic compounds (PDMS, passive sampling, GC-MSMS):
        - Polycyclic aromatic hydrocarbons (PAH)
        - Organochlorine pesticides (OCPs)
        - Organophosphate ester (OPE)
        - Pyrethroids
    - Volatile organic compounds (Tenax, passive sampling, ATD-GCMS):
        - Volatile phthalates
        - Volatile PAHs
        - Volatile OPEs
        - Organic acid anhydrids
        - Aldehydes/ketones
        - Benzene, toluene, etylbenzene and xylene (BTEX)
        - Phenols
        - Solvents
        - Alcohol/disinfectants
        - Microbial volatile organic compounds

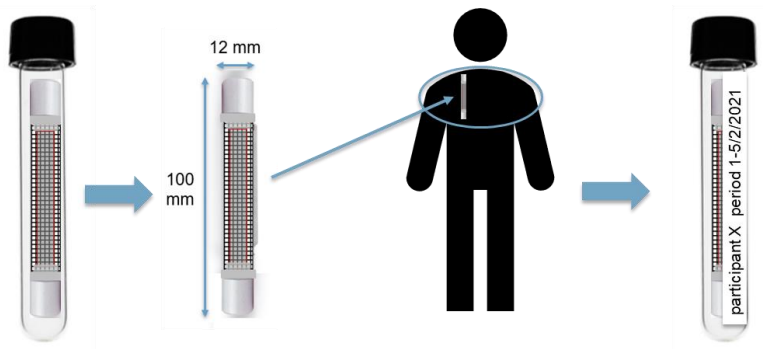

Figure 5. *The pen sized passive sampler in its transport tube (left and right) and exposed to ambient air (second from right) (source: from the WP1 Feasibility study protocol, Appndix S).*

- Sensor system with sensor box (EPHOR), activity sensor (AXIVITY) and heart rate monitor (POLAR) - as default sensors (50 sensors systems to be distributed among the centers). A feasibility study organized by WP1 will be performed in the period June – September 2021 in order to test and optimize the sensor system.The sensor system will be worn a full work week both at work and outside work where possible (source: WP1, **Appendix T)**:
    - Sensor box for measuring physical exposures (EPHOR, VTEC, Eidhoven, the Netherlands). The sensor will be worn on the body (uncovered), with a measurement frequency of 1 time/minute. The sensor will measure:
        - particulate matter (PM), including PM_1, $PM_{2.5}$ and PM_{10} ($\mu g/m^3$)
        - light intensity (lux)
        - UV intensity (W/cm^3)
        - temperature (°C)
        - relative humidity (%)
        - sound (dB(A))
    - Activity tracker (Ax3, Axitivity Ltd, UK): measures activity (physical activity), sleep and skin temperature. Time of measurement and charging "to be decided" after feasibility study.
    - Heart rate monitor (Polar H10, Polar): measures beats per minute, timing and downloading to "to be decided upon" after feasibility study.

- Biomarkers of chemical exposure (internal exposures) in blood and urine. Blood and urine will be collected at baseline (Monday morning) and at follow-up (Friday morning). Due to constraints in funding we will prioritize to analyse the follow-up samples, while the remaining 350 samples will be stored in the freezer. The following biomarkers of exposure will be measured:
    - Blood: lead (RBC) by ICP/IMS-screening (source: EPHOR WP3, for collection and handling of blood, **Appendix M2**)
    - Urine (source: EPHOR WP3, for collection and handling of urine, **Appendix N2**):
        - Metals (arsenic and cadmium) using ICP/MS- screening
        - PAH, measured as the pyrene-metabolite 1-hydroxypyrene (1-OHP)
- Residential exposure to dust for analysis of microbiome diversity and abundance, including the airborne dust settling on the surface measured by an electrostatic dust fall collector (EDC) (source: ECHRS IV, **Appendix U**)

B.2 Outcomes

The outcomes of the short-term study are acute respiratory effects based on changes in symptom scores, changes in lung function and altered level of short-term biological effect markers:

- Weekly change in lung function, measured as pre-bronchodilator FEV_1 and FVC (source: ECRHSIV clinical protocol, **Appendix C**)

- Daily change in lung function, measured as pre-bronchodilator FEV_1 and FEV_6 (source: **Appendix V**)
- Day-to-day change in respiratory symptoms measured on a visual analogue scale. Respiratory symptoms will be collected using the "How am I" App (developed by project partners at TNO, (source: App questions WP1, questions 4-5 + 20-26, **Appendix R**)
- Weekly change in respiratory symptoms measured on a visual analogue scale at the beginning and end of the week at the visit to the study clinic (source: "How am I" App questions WP1, questions 4-5 + 20-26, **Appendix R**)
- White blood count (WBC) including total numbers of peripheral leukocytes, lymphocytes, neutrophils, eosinophils, basophils and monocytes. The centers can choose between using the point-of-care testing system HemoCue (HemoCue AB, Ängelholm, Sweden, **Appendix M3**) or conventional analysis in a laboratory close to the research clinic (source: EPHOR WP3, for collection and handling of blood, **Appendix M2**)
- Short-term effect biomarkers in blood will be measured in blood (all inflammatory markers) to study how occupational exposures impact the associations between inflammatory markers and acute changes in lung function[1] (source: EPHOR WP3, for collection and handling of blood, **Appendix M2**). Blood samples will be collected at baseline (Monday morning) and at follow-up (Friday morning) for all participants. We are aiming at analyzing both samples (Monday and Friday) for all participants, but the analysis will depend on funding. When selecting samples to be analyzed it will be important to take into account potential bias (e.g. only follow-up samples or half of the participants both at baseline and follow-up). The biomarkers will be analysed using a Luminex technique (ISGlobal, Barcelona, Spain):
    - Interleukines (IL): IL-1β, 2, 4, 5, 6, 8, and 10
    - TNF-α
    - High sensitivity C-reactive protein (hsCRP)
    - Serum Ameloid A (SAA)

B.3 Potential confounders

- Age (source: ECRHSIII and Constances)
- Sex (source: ECRHSIII and Constances)
- Smoking assessed by measurements of the biomarker cotinine in blood[2] (source: EPHOR WP3, for collection and handling of blood, see **Appendix M2**) and from the interview in the long term study (source: ECRHSIII/IV main interview questionnaire, **Appendix A**, question 70)
- Previous exposures (to be defined)
- Health status (source: ECRHSIII/IV main interview questionnnaire, question 85, **Appendix A**, co-morbidity; Constances baseline questionnaire)

[1] The interpretation of the findings will be based on an internal comparisons of levels between participants by e.g. exposure and health status. We will probably use the overall mean or median cytokine level as a reference cut-off to identify comparatively abnormal cytokine levels in subgroups.
[2] Inclusion of analysis of cotinine in urine will be discussed when further planning the analysis strategy.

B.4 Potential effect modifiers

- Age (source: ECRHSIII and Constances)
- Sex (source: ECRHSIII and Constances)
- Atopic disposition (family history of atopic disease) (source: ECRHSIII main interview questionnnaire, **Appendix A**)
- Atopic status, determined by elevated specifc IgE antibodies in serum, by patch testing, or by interview information on hay fever (source: ECRHSIII main interview questionnnaire, **Appendix A**; Constances, questionnaire information)
- Biological markers of susceptibility to respiratory disease, all measured in serum at baseline (Monday morning) (n=400):
    - YKL40 and CC16
    - ROS damage: hydroxydeoxyguanosine (8-OHdG)

B.5 Analyses of biological pathways

The abundant information and biological material collected in the short-term study allow analysis of biological pathways. In addition to blood and urine, the following biological material will be collected Monday and Friday morning and analysed:

- Exhaled air can contain volatile and nonvolatile particles and aerosols. In EPHOR, the following will be collected:
    - **Exhaled breath (EB)** will be collected over approx. 10 minutes (normal breathing) using ReCIVA breath sampler mask. VOCs concentrated on absorbent tubes (endogenous metabolites and exogenous chemicals) for storage an later analysis by GC-MS (source: EPHOR WP3 (Owlstone), **Appendix W**).
    - **Exhaled breath condensate (EBC)** will be collected for **all participants** (n=400) using a freezing cooling chamber (TurboDECCS system, Medivac SRL, Parma, Italy) to cool and condense the exhaled breath. The method requires 15 minutes of tidal breathing (1 – 2 mL of EBC). EBC will be used for analysis of cfDNA methylation and proteomics (source: EPHOR WP3 (KU Leuven), **Appendix X**). A protocol for collection, pre-processing, storage and analysis of exhaled breath condensate for assessment of cfDNA methylation and proteomics, will be developed during the course of the project and made available after validation.
    - **Respiratory droplets (RB)** are droplets (proteins, viral DNA/RNA and particles, drugs, non-volatile metabolites) collected on filter over 30 minutes for storage and later analysis by ELISA or LC-MS. EBA targets is focused on immune-related proteins (e.g. CRP, interleukins, etc.). The collection requires breathing manoeuvres (coughing, speaking, forced exhalation) (source: EPHOR WP3 (Owlstone), **Appendix Y**)
        - **Note 1:** EB and RB will be collected from the same 220 participants (at the beginning and end of the week = 440 samples) and in few centers as the procedure requires a breath collection station with running costs and site variation.

Table 2 *Overview over data and samples collected in the short-term study (existing/biobanked material is not included). X = information or data collected where number indicates times per day, C = sample being collected and stored, A = sample being analyzed in EPHOR (funded).*

Data and material to be collected	Day 1	Day 2	Day 3	Day 4	Day 5
Baseline participant info					
Baseline and screening questionnaire	X				X
App questions*	2X	2X	2X	2X	2X
Lung function					
EasyOne (FEV_1, FVC)	X**				X***
Vitalograph micro (FEV_1, FEV_6*)*	3X	3X	3X	3X	X
WP1 Sensor system					
Sensor 1, passive sampler, chemical exp.	→	→	→	→	
Sensor 2, box, physical exp.	→	→	→	→	
Sensor 3, heart rate	→	→	→	→	
Sensor 4, activity	→	→	→	→	
Electrostatic dust fall Collector (EDC) for residential exposure to dust**	→	→	→	→	→
Biological sample collection					
Blood	X				X
Metals (Pb, As) (maybe Cd, Hg, Pt, Fe, Mn)					**A**
Cotinine (nicotine, smoking)[3]	**C**				**A**
Differential blood count	**A**				**A**
Inflammation markers (proteomics) (CRP, SSA, interleukins, TNF-α, etc)	**C**				**A**
Inflammation markers (YKL-40 + CC-16)	**C**				**A**
ROS-damage (8-OHdG)	**C**				**A**
Telomere length/mtDNA	**C**				**A**
Epigenomics *- Array epigenomics in blood DNA (EWAS)* *- Pyrosequenzing (SNPs)* *- cfDNA metylation*	**C**				**A**
Transcriptomic analysis (targeted), ddPCR	**C**				**A**
qPCR (expression of a set of 10-15 genes)	**C**				**A**
Urine					
Metals (cadmium, arsenic)					**A**
1 -hydroxypyrene (PAH)	**C**				**A**
Exhaled breath samples					
Exhaled breath (EB) - *VOCs*	**C**				**A**
Exhaled Respiratory droplets (RD) *- Proteomics*	**C**				**A**
Exhaled Breath Condensate (EBC) *- Proteomics and cfDNA methylation*	**C**				**A**

* Respiratory symptoms, wellbeing, sleep, physical activity, working time, use of PPE and commuting, ** = without reversibility testing, *** = including reversibility-testing, **** = Microbiome diversity and abundance

[3] Inclusion of analysis of cotinine in urine will be discussed when further planning the analysis strategy.

4. Management of data

Data flow of existing data and biological material in the long-term study

The flow of existing data in the EPHOR WP6 long-term study is illustrated in **Figure 6**. We will use data collected in 2010-2012 in the ECHRS cohort and in 2018-2020 in the Constances cohort. Data from questionnaires and lung function tests collected in both ECHRS and Constances are currently located on three different databases in Norway, Spain and France. These data will be transferred to the YODA platform in the Netherlands, hosted by UU. WP4 is responsible for coordinating this. Blood samples have previously been collected in both the ECHRS cohort and Constances. The ECRHS blood samples are currently stored in Norway, Australia and Spain and will be sent to ISGlobal in Spain, where they will be analysed. The blood samples from Constances are currently stored at IBBL in Luxemburg and will also be transferred to ISGlobal for analyses. The results from the blood measurements will be transferred to the YODA platform. WP3 is responsible for the coordination and analyses of the blood samples.

The combined data (questionnaires, lung function and analyses results of blood samples) will be accessed from YODA and analysed directly on the YODA and analyzed at YODA related platforms for scientific publications, and an EU report about the data collection will be prepared.

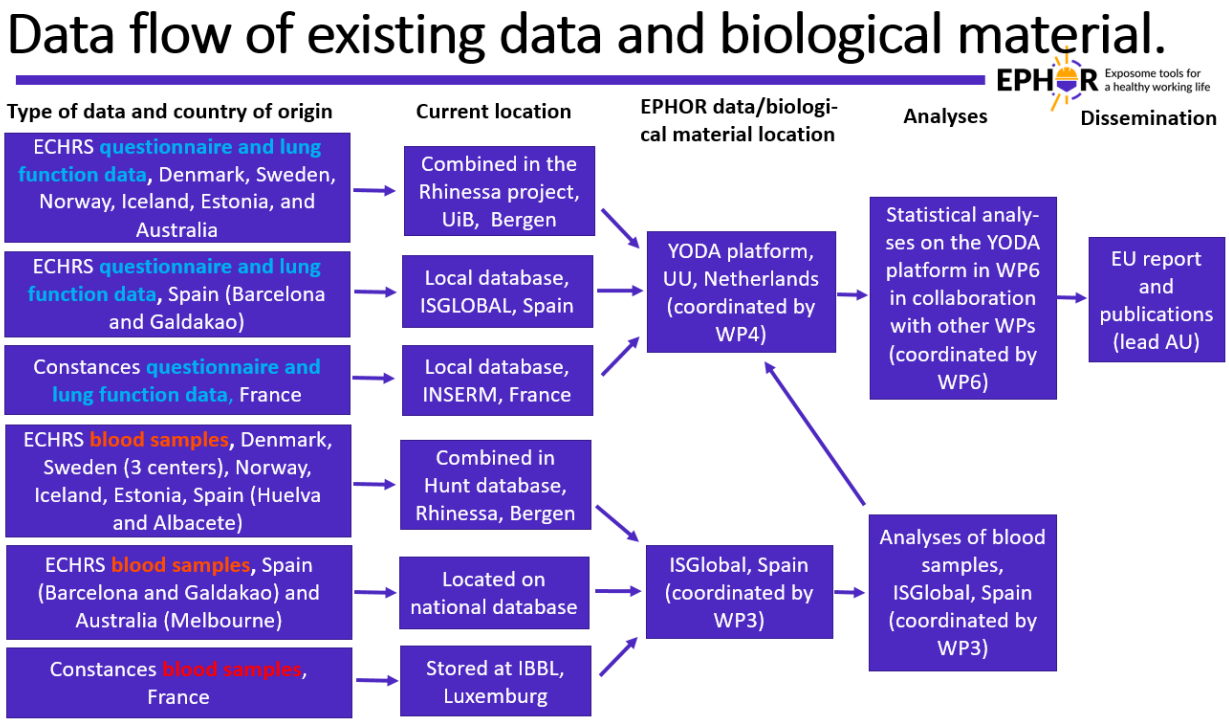

***Figure 6**. Data flow of the existing data and biological material in the long-term study in WP6.*

Flow of new data and biological material collected in the short- and long-term studies

The flow of the collection of new questionnaire, clinical and environmental data and biological material in the EPHOR WP6 long- and short-term studies is illustrated in **Figure 7**. All participating study centers will collect data and biological material locally and this will be transferred to AU (WP6 lead), Denmark. AU is responsible for handling the data or transfer of data and biological material to the EPHOR partners that will manage the data and material processing. AU will transfer all data from questionnaires, lung function tests and other clinical examinations to the YODA platform in the Netherlands, hosted by UU. The information collected in the app will be processed at TNO, Netherlands, and then uploaded to the YODA platform. The data from the sensor boxes and passive samplers will be processed at TNO, Netherlands and the results uploaded to YODA. AU is responsible for processing of the electrostatic dust fall collectors and will upload the results to YODA. The blood, urine, exhaled breath, exhaled breath condensate and respiratory droplets will be analysed by KU Leuven (WP3) or by EPHOR partners or sub-contractors. WP3 is responsible for coordinating the analyses of the biological material and for uploading the results to the YODA platform. After finalizing the analyses of blood, urine, exhaled breath, exhaled breath condensate, respiratory droplets and electrostatic dust fall collectors, the material will be destroyed or sent back for biobanking in the study centers, where they were collected. The combined data (questionnaires, lung function and other clinical examinations and analyses results of blood and urine samples in addition to exhaled breath sample and electrostatic dust fall collectors) will be accessed from the YODA platform and analyzed at YODA related platforms for scientific publications, and an EU report about the data collection will be prepared.

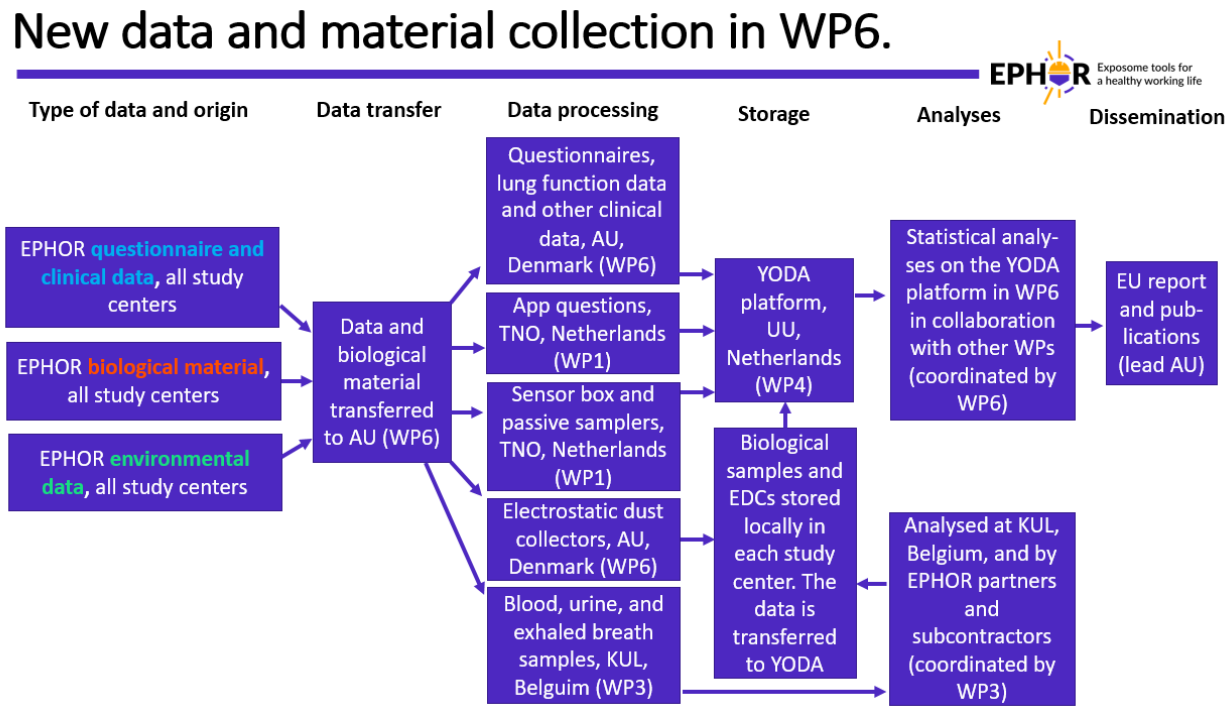

***Figure 7**. Data flow of the new data and material collection in the short- and long-term studies in WP6.*

5. Statistical analysis and study power

Statistical power

Long-term study

In the follow-up study of the ECRHS cohort, information is collected from all former participants, who wish to participate. We expect that 3000 participants from the included study centres will consent to participate in the follow-up study. Based on previous studies, including assumptions about exposure levels and a mean (SD) annual decline in FEV_1 of 43 (26) mL in population studies (Fuertes et al. 2018), using linear regression with a statistical power of 96%, we will be able to detect a further decline in FEV_1 of at least 15 mL per year.

Short-term study

In the short-term study, we hope to include 400 subjects among all participating study centers. We assume the following: (i) mean (SD) occupational exposure to dust of 1 (2) mg/m^3 (Schlünssen et al. 2004) and an expectation of a similar distribution of other occupational exposures, (ii) a mean (SD) FEV_1 of 3.5 (0.8) L among people with asthma in population studies (Boudier et al. 2019), (iii) that the strength of the association between short-term exposure and change in FEV_1 is within the same range for all exposures, and (iv) that the proportion of non-valid observations is less than 9%. Using linear regression, with a power of 80% and 3200 observations (2 observations/day * 4 days/participant *400 participants), we would detect a change in FEV_1 of at least 22 mL per unit exposure (Schlünssen et al. 2004). However, we plan that participants will perform three daily self-measurements of lung function, and not two as used in the power calculations. In addition, the power calculation is based on independent measurements and is therefore a very conservative estimate, since we have paired measurements. We therefore expect to have sufficient power.

Statistical analysis

General considerations

For the data analyses, we will use both established methods and new methods under development within the EPHOR project. In particular, the analyses will follow an exposome concept and focus on the association between multiple exposures and respiratory outcomes. As a starting point, we will use more conventional methods in order to analyse all associations between exposures and outcomes in parallel, while adjusting for multiple testing. We will apply new methods using clustering of variables to give profiles of different groups potentially related to the outcome of interest, where it would be possible to consider all exposure variables in a unique model that takes into account correlations between the multiple exposures, for example methods like the Bayesian profile regression (Guillien et al. 2021). We will also apply machine learning techniques including text mining and bioinformatics omics analyses in order to inform the epidemiological analyses. The applied methods will also include relevant and extensive interaction and pathways analyses between exposures and between exposures and genes, gene transcripts and proteins.

Considerations for the long-term study

In the long-term study, we aim to assess the joint effects of all measured occupational exposures (classical: airborne / non-classical: psychosocial, light, UV, noise) on a) decline in lung function, b) incidence of asthma and COPD and c) severity of asthma and COPD. For all outcomes, we will assess dose-response relationships. We will address how much variation in outcome can be explained by a) classical exposures and b) non-classical exposures. We will investigate exposure window of relevance for asthma (cumulative / recent / highest attained exposure (peak)). For COPD, we hypothesize that cumulative exposures is the exposure window of relevance, but we will also explore other exposure windows as well as exposure lags. We will assess the impact of early life factors (e.g. infections, passive smoking, atopic disposition), as well as impact of age and gender.

We hypothesize that the joint effect of the general external exposome (air pollution) and the specific external exposome (high occupational exposure, certain diets, low physical activity, tobacco smoking) will increase the risk for accelerated lung function decline, (exacerbation of) COPD and (exacerbation of) asthma. Furthermore, we hypothesize a larger risk for high occupational and high air pollution levels among subjects with high YKL40 and low CC16 levels at baseline.

A key research question in the long-term study is how the susceptibility markers YKL40 and CC16 modify the association between exposure and outcome. A first simple approach to investigate potential effect modification would be to use stratification by YKL 40 and CC16 levels dichotomized, and a similar approach can be used for gender and age. More advanced approaches will also be adapted, for example a multilevel model, a random forest model or a Bayesian analysis with priors in order to take into account the different effects and their interactions.

Considerations for the short-term study

In the short-term study, we aim to address the joint effects of all measured occupational exposures (classical: airborne / non classical: light, UV, noise) on acute decline in lung function and symptom severity, and we will address how much variation in outcome can be explained by a) classical exposures and b) non classical exposures. We hypothesize that the effect of the occupational exposome on change in symptom score (or lung function) cross-week is partly mediated through an inflammatory cascade and modified by susceptibility factors. Furthermore, we hypothesize that the joint effect of the specific external exposome (measured by sensors and passive sampling), other specific external exposome (certain diet, low physical activity, tobacco) and the internal exposome (exposure biomarkers) will increase the risk for cross-day and cross-week lung function decline/severity in symptoms and the level of inflammatory markers and ROS.

In the short-term cross week study, we will be able to investigate changes in exposures and changes in the outcomes (i.e. lung function and asthma symptoms) in addition to changes in multiple biomarkers assessed by various omics techniques. Using pathway analyses, we will be able to analyse pathways between

a) (change in) exposures – (change in) inflammatory markers – (change in) symptoms/lung function.

b) (change in) exposures – (change in) methylation – (change in) protein expression – change in symptoms/lung function.

Furthermore, using mediation analysis, we will estimate the contribution of both direct effects (not mediated by inflammatory markers) and indirect effects (mediated by inflammatory markers) on the association between exposures and lung function/symptoms (**Figure 8**). Finally, the high-resolution data collected in the short-term study allow us to investigate time-dependent associations between peak exposures and lung function and asthma symptoms.

We will prioritize exposures when the final study population is known, including the distribution of job titles and the most prevalent exposures. Having this specific information will enable us to identify biomarkers that are especially relevant to the most prevalent job titles and exposures.

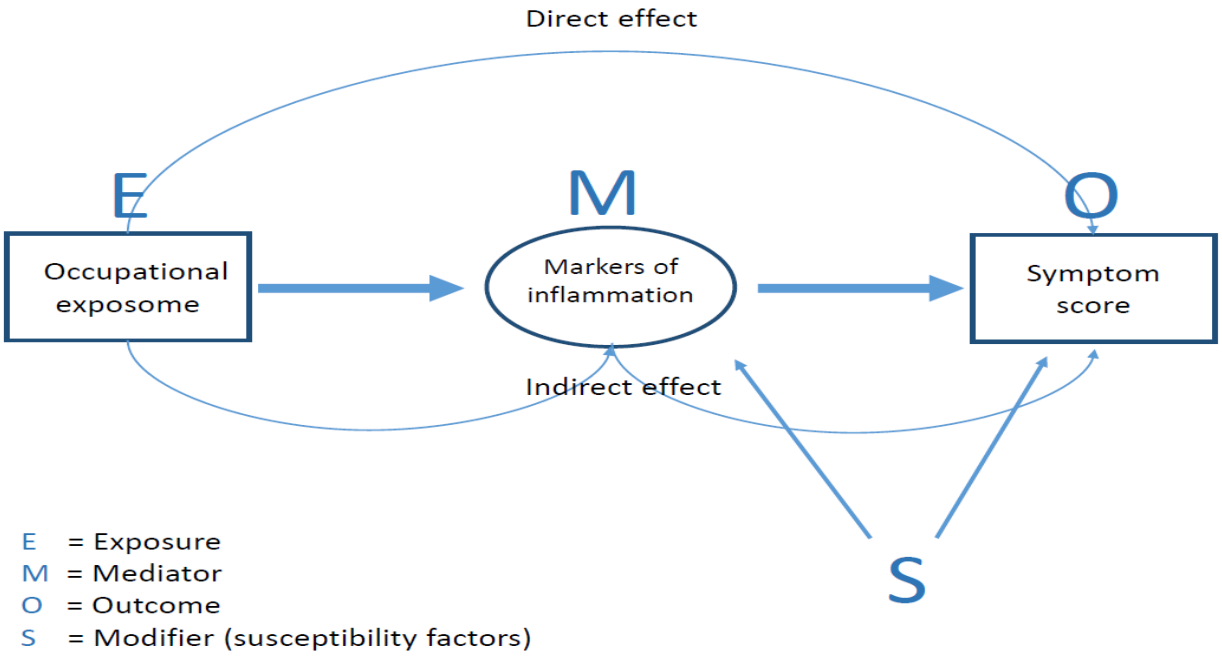

***Figure 8**. Model illustrating the analyses of direct and indirect pathways in the analyses strategy for the short-term study.*

6. Ethical issues

Issues on informed consent procedures, informed sheets and consent forms, update of opinions/approvals by ethics committees and/or competent authorities for the research on humans for the participating centers, as well as details on the pertinent and incidental findings policy is addressed in "Deliverable D13.9" (due date Month 18), but is summarized below.

Informed consent procedures, including recruitment, inclusion and exclusion criteria, are described above. In general, the procedure for informing the patients will differ somewhat between the centers, depending on how they are recruited. However, all subjects will be provided

written and oral information about the study in the local languages prior to enrollment. For the existing data both cohorts have consent forms where participants have consented to participate in the research projects and consent for data processing.

Informed sheets and consent forms: Three variants of information sheets and consent forms will be used in EPHOR WP6 depending on the cohort (ECRHS or Constances Cohort) or whether the short- and long-term study are applied for separately (e.g. Norway and France) or at the same time (Denmark). The information sheets and consent forms will be tailored to the cohort and population in question.

Opinions/approvals by ethics committees: The protocol for EPHOR WP6 and the associated questionnaires are still under revision in order to be finalized for the next follow up in ECRHSIV and submission within the deadline for deliverable D6.1 (month 18). Hence, since most centers/countries need the protocol to be finalized before applying REC, the applications for ethical approval will not be submitted for all centers within the deadline of this deliverable (D13.9, 18 months). In brief, for ECRHS, two out of a total of nine ECRHS-centers will be ready to collect data autumn 2021 (Denmark and Norway).

Incidental findings policy: An **incidental finding** is 'a finding concerning an individual research participant that has potential health or reproductive importance and is discovered in the course of conducting research but is beyond the aims of the study'. It is often referred to three requirements that should be fulfilled prior to reporting incidental findings back to research participants (Wolf et al. 2012;Dyke et al. 2019). Findings should be:

- analytically valid
- clinically significant
- actionable

EPHOR WP6 contains and collects extensive data on respiratory health, occupational and environmental exposure including life style factors, as well as collection of biological material that will be analysed for a range of biomarkers of exposure and effect. This makes it necessary to evaluate and decide on what factors should be considered when determining whether incidental findings should be reported to participants.

Based on a thorough review of the information and biological data collected and processed in EPHOR WP6, the following findings has been defined as "Incidental findings":

- Lead in blood substantially outside the normal range
- White blood cell (WBC) count substantially outside the normal range and abnormal WBC distribution
- C-reactive protein (CRP) substantially outside the normal range
- Abnormal heart rate (arrythmia) that can not be explained by known disease, physical activity, etc.

Other issues

Both females and males, and non-smokers and smokers, are included in the study.

EPHOR WP6 aims, together with partners from WP3, at developing non-invasive methods for collection of biological material as alternative matrixes for blood (e.g. exhaled breath condensate, respiratory droplets).

Blood will be collected using standard venesection techniques. Staff should be trained and insured to carry out venepuncture according to local requirements.

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8. Appendixes

Overview of protocols - questionnaires and standard operating procedures

Long-term study

Participants recruited to the **long-term study** from ECRHS will be invited to the local testing centre for the following questionnaires and clinical investigations which are listed below in the proposed order (to be determined, below proposal based on ECRHS).

- Explanation of procedure and consent
- Short Screening Questionnaire (ECRHSIV, version 5.0, Appendix 0)
- Main questionnaire (interview, ECRHSIV, Appendix A)
- Getting ready for FENO, bioimpedence, spirometry, reversibility, questionnaire
- FeNO (Appendix L)
- Height, Weight, Waist and Hip circumference and Bioimpedence (Appendix D)
- Collection of microbiome/gingival fluid (Appendix O)
- Lung function testing (with reversibility) (Appendix C)
- Completion of SF-36 (Appendix H), AQLQ (Appendix I), Woman's questionnaire (Appendix J) and Male's questionnaire (Appendix K) while waiting for bronchodilator (salbutamol) effect
- Food frequency questionnaire **check** (self-administred at home) (Appendix E)
- Exposure to sunlight questionnaire (Appendix B)
- *It may be appropriate to perform venesection at this moment*
- Post bronchodilator measure of lung function (Appendix C)
- Collection of saliva fluid (Appendix P)

There may be differing local challenges regarding the order in which these components are performed depending on local facilities. However when organising your local **long-term study** the following rules should be considered:

1. The following questionnaires MUST be self-completed:
    - Food frequency questionnaire
    - Exposure to sunlight questionnaire
    - SF-36
    - AQLQ
    - Woman's questionnaire and Male's questionnaire

2. The SF-36 and AQLQ **MUST** be completed AFTER the main questionnaire
3. Post bronchodilation FEV_1 **MUST** be read AT LEAST 15 minutes after administration of bronchodilator
4. Where local permission is given the "Food frequency questionnaire (FFQ)" and the "Exposure to sunlight questionnaire" may be sent with the invitation letter to the testing centre or with the details of how to get to the testing centre, completed by the individual at home and checked by a fieldworker in the clinic for completeness.

Short-term study

The order of the questionnaires, sensor systems and clinical investigations for the **short-term study** will be determined based on an ongoing pilot study in WP3.

Appendix 0. Short Screening Questionnaire ECRHS IV, Version 5.0

TO ANSWER THE QUESTIONS PLEASE MAKE A TICK IN THE APPROPRIATE BOX
IF YOU ARE UNSURE OF THE ANSWER PLEASE CHOOSE 'NO'

1. Have you had wheezing or whistling in your chest at any time in the last 12 months? NO ☐ YES ☐

If 'NO' go to question 2….. If 'YES':

1.1. Have you been at all breathless when the wheezing noise was present? NO ☐ YES ☐

1.2. Have you had this wheezing or whistling when you did not have a cold? NO ☐ YES ☐

2. Have you woken up with a feeling of tightness in your chest at any time in the last 12 months? NO ☐ YES ☐

3. Have you been woken by an attack of shortness of breath at any time in the last 12 months? NO ☐ YES ☐

4. Have you been woken by an attack of coughing at any time in the last 12 months? NO ☐ YES ☐

5. Have you had an attack of asthma in the last 12 months? NO ☐ YES ☐

6. Are you currently taking any medicine (including inhalers, aerosols or tablets) for asthma? NO ☐ YES ☐

7. Do you have any nasal allergies including hay fever? NO ☐ YES ☐

8. Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last 12 months? NO ☐ YES ☐

9. Have you had an attack of shortness of breath that came on following strenuous activity at any time in the last 12 months? NO ☐ YES ☐

10. Have you **ever** had asthma NO ☐ YES ☐

If 'NO' go to question 11…… If 'YES' -

10.1. How old were you when you had your first attack of asthma? YEARS ☐☐

(if started as a baby enter '01)

10.2. Was this confirmed by a doctor? NO ☐ YES ☐

11. Do you cough on most days for as much as three months a year? ☐ ☐

12. Do you bring up phlegm from your chest on most days for as much as three months a year? NO ☐ YES ☐

13. Have you ever been told by a doctor that you had chronic obstructive pulmonary disease or emphysema? NO ☐ YES ☐

If 'NO' go to question 14…… If 'YES' –

13.1. How old were you when you were told that you had chronic obstructive pulmonary disease or emphysema? YEARS ☐☐

14 In the last 12 months, have you regularly (on most days) taken any of the following inhalers or any other **STEROID** inhaler? NO ☐ YES ☐

AeroBec	Asmabec	Alvesco	Asmanex	Pulmicort
Beclometasone	Becodisks	Beclazone	Budesonide	Symbicort
Clenil	Fostair	Flixotide	Qvar	Seretide

15. Have you ever smoked for as long as a year? NO ☐ YES ☐

If 'NO' go to question 16……. If 'YES'

15.1. Have you smoked in the last month? NO ☐ YES ☐

15.2 How old were you when you last smoked? YEARS ☐☐

16. How many years have you lived in your current home? *(If less than 12months enter '01')* YEARS ☐☐

17. How old are you? YEARS ☐☐

18. What is today's date? DAY ☐☐ MONTH ☐☐ YEAR ☐☐

19. Please can you write your postcode clearly here POSTCODE ☐☐☐☐ ☐☐☐

COVID (coronavirus) AND YOUR LUNG HEALTH

20. Have you been vaccinated against COVID (coronavirus)? NO ☐ YES ☐

If 'NO' - go to Question 21……. If 'YES' -

20.1 On what date did you have your first vaccination? DAY ☐☐ MONTH ☐☐ YEAR ☐☐

(make your best guess if you don't know precisely)

NO YES

21. Do you think you have had COVID (coronavirus) ☐ ☐

If 'NO' - you have completed this questionnaire
If 'YES' - please answer the following questions

21.1 What makes you think you have had COVID (coronavirus)?	NO	YES
21.1.1 I had a positive test	☐	☐
21.1.2 A doctor/nurse told me I had it	☐	☐
21.1.3 I just think I must have had it	☐	☐

21.2 . On what date do you think your COVID (coronavirus) infection began? *(make your best guess if you don't know precisely)*

DAY ☐☐ MONTH ☐☐ YEAR ☐☐

21.3 What symptoms did you experience? Please answer each question	NO	YES
21.3.1 Fever/high temperature	☐	☐
21.3.2 Headache	☐	☐
21.3.3 Fatigue/Tiredness	☐	☐
21.3.4 Muscle aches/myalgia	☐	☐
21.3.5 Cough	☐	☐
21.3.6 Shortness of breath or difficulty breathing	☐	☐
21.3.7 Blocked or runny nose	☐	☐
21.3.8 Loss of sense of smell and/or taste	☐	☐
21.3.9 Nausea or vomiting	☐	☐
21.3.10 Diarrhoea	☐	☐
21.3.11 Chest pains	☐	☐
21.3.12 Skin rashes	☐	☐
21.3.13 Other – free text_____	☐	☐

21.4 <u>After the acute symptoms of COVID ended</u>, have you experienced **<u>MORE</u>** of any of the following breathing problems <u>when compared to your breathing **BEFORE** you had COVID</u>?

	No change	*More since I had COVID*
21.4.1 wheezing or whistling in your chest	☐	☐
21.4.2 waking with a feeling of tightness in your chest	☐	☐
21.4.3 waking with attacks of shortness of breath	☐	☐
21.4.4 waking with attacks of coughing	☐	☐
21.4.5 attacks of asthma	☐	☐
21.4.6 shortness of breath when hurrying on level ground or walking up a slight hill	☐	☐

<u>Please write here</u> if there is anything else you think we should know about your respiratory health

_____

_____

_____

THANK YOU FOR YOUR HELP:
If you don't mind being telephoned at home or at work by one of the study team, please write your telephone number below:

(DAY).....**(EVE)**.....

(EMAIL) .....

PLEASE CONTACT THE STUDY TEAM ON 020 XXXX IF YOU ARE UNABLE TO COMPLETE THE WRITTEN QUESTIONNAIRE AND WOULD LIKE ASSISTANCE CO

Appendix A. ECRHS IV main interview questionnaire *(in draft, 23.06.21)*

ECRHS IV– Interviewer Administered Questionnaire

Centre number ☐☐☐
Personal number ☐☐☐☐☐
Sample ☐
Date ☐☐ ☐☐ ☐☐

I AM GOING TO ASK YOU SOME QUESTIONS. AT FIRST THESE WILL BE MOSTLY ABOUT YOUR BREATHING. WHEREVER POSSIBLE, I WOULD LIKE YOU TO ANSWER 'YES' OR 'NO'.

1. Have you had wheezing or whistling in your chest at any time in the last ***12 months***? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 2, IF 'YES':

1.1 Have you been at all breathless when the wheezing noise was present? NO ☐ YES ☐

1.2 Have you had this wheezing or whistling when you did ***not*** have a cold? NO ☐ YES ☐

1.3 How old were you when you first had wheezing or whistling in your chest? YEARS ☐☐

1.4 How frequently have you had wheezing or whistling in the last 12 months? TICK ONE BOX ONLY

Everyday	1 ☐
at least once a week, but not everyday	2 ☐
Occasionally	3 ☐

2. Have you woken up with a feeling of tightness in your chest at any time in the last ***12 months***? NO ☐ YES ☐

3. Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last ***12 months***? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 4, IF 'YES':

3.1 How old were you when you first had an attack of shortness of breath that came on during the day when you were at rest? YEARS ☐☐

4. Have you had an attack of shortness of breath that came on ***following*** strenuous activity at any time in the last ***12 months***? NO ☐ YES ☐

5. Have you been woken by an attack of shortness of breath at any time in the last ***12 months?*** NO ☐ YES ☐

6. Have you been woken by an attack of coughing at any time *in the last* ***12 months***? NO ☐ YES ☐

7. How often have you experienced bouts or spasms of coughing in the last 12 months? TICK ONE BOX ONLY

less than once a month	1 ☐
every month, but less than every week	2 ☐
every week, but not every day	3 ☐
every day	4 ☐

	NO	YES
8. Do you ***usually*** cough first thing in the morning in the winter? **[IF DOUBTFUL, USE QUESTION 9.1 TO CONFIRM]**	☐	☐
9. Do you ***usually*** cough during the day, or at night, in the winter?	☐	☐

IF 'NO' GO TO QUESTION 10, IF 'YES':

9.1 Do you cough like this on most days for as much as three months each year?	☐	☐

	YEARS
9.2 How many years have you had this problem (coughing on most days for as much as three months each year)?	☐☐

10. Do you ***usually*** bring up any phlegm from your chest first thing in the morning in the winter? **[IF DOUBTFUL, USE QUESTION 11.1 TO CONFIRM]**	☐	☐
11. Do you ***usually*** bring up any phlegm from your chest during the day, or at night, in the winter?	☐	☐

IF 'NO' GO TO QUESTION 12, IF 'YES':

11.1 Do you bring up phlegm like this on most days for as much as three months each year?	☐	☐

	YEARS
11.2 How many years have you had this problem (of bringing up phlegm from your chest on most days for as much as three months each year)?	☐☐

IF 'NO' TO QUESTIONS <u>1-11</u> GO DIRECT TO QUESTION 13;
IF 'YES' TO ANY OF QUESTIONS <u>1-11</u> PLEASE COMPLETE QUESTION 12:

12. In the last ***12 months***, have you had any episodes/times when your symptoms (cough, phlegm, shortness of breath) were a lot worse than usual?	☐	☐

IF 'NO' TO QUESTION 12 GO TO QUESTION 13; IF 'YES':

In the last ***12 months***:	TIMES
12.1 How many times have these episodes occurred?	☐☐
12.2 How many times have these episodes forced you to consult your doctor?	TIMES ☐☐
12.3 How many times was your therapy changed after these episodes?	TIMES ☐☐
12.4 How many times have you visited a hospital casualty department or emergency room or have you spent a night in hospital after these episodes?	TIMES ☐☐

13. Do you ever have trouble with your breathing? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 14, IF 'YES':

13.1 Do you have this trouble — TICK ONE BOX ONLY

a) continuously so that your breathing is never quite right? 1 ☐

b) repeatedly, but it always gets completely better? 2 ☐

c) only rarely? 3 ☐

14. Are you disabled from walking by a condition ***other than*** heart or lung disease? NO ☐ YES ☐

IF 'YES' STATE CONDITION _____ AND GO TO QUESTION 15, IF 'NO':

14.1 Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 15, IF 'YES':

14.2 Do you get short of breath walking with other people of your own age on level ground? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 15, IF 'YES':

14.3 Do you have to stop for breath when walking at your own pace on level ground? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 15, IF 'YES':

14.4 Do you ever have to stop for breath after <u>walking about 100 yards</u> (or after a few minutes) on <u>level ground?</u> NO ☐ YES ☐

IF 'NO' GO TO QUESTION 15, IF 'YES':

14.5 Are you too short of breath to leave the house OR short of breath on dressing or undressing? NO ☐ YES ☐

15. Have you ever had asthma? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 16, IF 'YES':

15.1 Was this confirmed by a doctor? NO ☐ YES ☐

15.2 How old were you when your asthma was confirmed by a doctor? YEARS ☐☐

15.3 How old were you when you had your first attack of asthma? YEARS ☐☐

15.4 How old were you when you had your most recent attack of asthma? YEARS ☐☐

15.5.1-6 Which months of the year do you usually have attacks of asthma?

	NO	YES
15.5.1 January / February	☐	☐
15.5.2 March / April	☐	☐
15.5.3 May / June	☐	☐
15.5.4 July / August	☐	☐
15.5.5 September / October	☐	☐
15.5.6 November / December	☐	☐

15.6 Have you had an attack of asthma in the last ***12 months***? NO ☐ YES ☐

IF 'NO' GO TO 15.9, IF YES

15.7 How many attacks of asthma have you had in the last ***12 months***? ATTACKS ☐☐

15.8 How many attacks of asthma have you had in the last ***3 months***? ATTACKS ☐☐

15.9 How many times have you woken up because of your asthma in the last ***3 months?*** TICK ONE BOX ONLY

every night or almost every night	1 ☐
more than once a week, but not most nights	2 ☐
at least twice a month, but not more than once a week	3 ☐
less than twice a month	4 ☐
not at all	5 ☐

15.10 How often have you had trouble with your breathing because of your asthma in the last ***3 months?*** TICK ONE BOX ONLY

continuously	1 ☐
about once a day	2 ☐
at least once a week, but less than once a day	3 ☐
less than once a week	4 ☐
not at all	5 ☐

15.11 Are you currently taking any medicines including inhalers, aerosols or tablets for asthma? NO ☐ YES ☐

15.12 Do you have a peak flow meter of your own? NO ☐ YES ☐

***IF 'NO' GO TO QUESTION 15.13, IF 'YES'*:**

15.12.1 How often have you used it over the last 3 months? TICK ONE BOX ONLY

never	1 ☐
some of the days	2 ☐
most of the days	3 ☐

15.13 Do you have written instructions from your doctor on how to manage your asthma if it gets worse or if you have an attack? NO ☐ YES ☐

16. Has a doctor ever told you that you have chronic bronchitis? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 17, IF 'YES':

16.1 How old were you when you first had a diagnosis of chronic bronchitis? YEARS ☐☐

17. Has a doctor ever told you that you have chronic obstructive pulmonary disease (COPD)? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 18, IF 'YES

17.1 How old were you when you first had a diagnosis of COPD? ☐☐ YEARS

18. Has a doctor ever told you that you have emphysema? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 19, IF 'YES':

18.1 How old were you when you first had a diagnosis of emphysema? YEARS ☐☐

19. Have you ever been diagnosed with any **other** lung disease (excluding asthma, chronic bronchitis, COPD and emphysema)? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 20, IF 'YES':

CODE

19.1 What is that lung disease called?_____ ☐☐

NO YES

20. Do you have any nasal allergies, including hay fever? ☐ ☐

IF 'NO' GO TO Q21, IF' YES':

YEARS

20.1 How old were you when you first had hay fever or nasal allergy? ☐☐

NO YES

21. Have you ***ever*** had a problem with sneezing, or a runny or a blocked nose when you did not have a cold or the flu? ☐ ☐

IF 'NO' GO TO Q22, IF 'YES':

NO YES

21.1 Have you had a problem with sneezing or a runny or a blocked nose when you did not have a cold or the flu ***in the last 12 months***? ☐ ☐

IF 'NO' GO TO Q22, IF' YES':

NO YES

21.1.1 Has this nose problem been accompanied by itchy or watery eyes? ☐ ☐

NO YES

21.1.2 In which months of the year did this nose problem occur?

21.1.2.1	January/February	☐ ☐
21.1.2.2	March/April	☐ ☐
21.1.2.3	May/June	☐ ☐
21.1.2.4	July/August	☐ ☐
21.1.2.5	September/October	☐ ☐
21.1.2.6	November/December	☐ ☐

NO YES

21.1.3 Have you had this problem for **more than 4 days in any one week** in the last 12 months? ☐ ☐

IF 'NO' GO TO Q21.1.4, IF' YES':

NO YES

21.1.3.1 Did this happen for **more than 4 weeks consecutively?** ☐ ☐

21.1.4. For **each** of the following problems, please indicate how important it has been ***over the last 12 months***. *(SHOW A CARD WITH THE FOLLOWING OPTIONS)*

1. No problem (symptom not present)
2. A problem that is/was present but not disturbing
3. A disturbing problem but not hampering day time activities or sleep
4. A problem that hampers certain activities or sleep

CODE

Please enter code 1-4 in each of the five boxes

21.1.4.1	a watery runny nose	☐
21.1.4.2	a blocked nose (feeling of being unable to breath through your nose)	☐
21.1.4.3	an itchy nose	☐
21.1.4.4	sneezing, especially violent and in bouts	☐
21.1.4.5	watery, red itchy eyes	☐

22. ***Since the last survey*** have you used any medication to treat nasal disorders? NO ☐ YES ☐

IF NO GO TO Q23, IF YES

22.1 Have you used any of the following nasal sprays for the treatment of your nasal disorder? **{SHOW LIST OF STEROID NASAL SPRAYS}** NO ☐ YES ☐

IF NO GO TO Q22.2, IF YES

22.1.1 How old were you when you first started to use **this sort of nasal spray**? YEARS ☐☐

22.1 2 How many years have you been taking this sort of nasal spray? YEARS ☐☐

22.1.3 Have you used any of these nasal sprays ***in the last 12 months?*** NO ☐ YES ☐

22.1.4. Have you used this sort of nasal spray ***every year*** in the last 5 years? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 22.2 IF 'YES'

22.1.4.1 On average how many months each year have you taken them ? MONTHS ☐☐

22.2 Have you used any of the following pills, capsules, or tablets for the treatment of your nasal disorder? **{SHOW LIST OF ANTIHISTAMINES}** NO ☐ YES ☐

IF 'NO' GO TO Q23, IF 'YES'

22.2.1 Have you used any of these pills, capsules or tablets in the last 12 months**?** NO ☐ YES ☐

23. Has your nose been blocked **<u>for more than 12 weeks during the last 12 months</u>?** NO ☐ YES ☐

24. Have you had pain or pressure around the forehead, nose or eyes **<u>for more than 12 weeks during the last 12 months</u>?** NO ☐ YES ☐

25. Have you had discoloured nasal discharge (snot) or discoloured mucus in the throat **<u>for more than 12 weeks during the last 12 months</u>?** NO ☐ YES ☐

26. Has your sense of smell been reduced or absent **<u>for more than 12 weeks during the last 12 months</u>?** NO ☐ YES ☐

27. Has a doctor ***ever*** told you that you have
- 27.1.1 **<u>chronic</u>** sinusitis? NO ☐ YES ☐
- 27.1.2 nasal polyps? NO ☐ YES ☐

IF 'NO' TO Q27.1 and 27.2 GO TO Q28, IF 'YES':

27.2 How old were you when a doctor told you had chronic sinusitis? YEARS ☐☐
27.3 How old were you when a doctor told you had nasal polyps? YEARS ☐☐
(enter 00 if question not applicable)

28. Have you ***ever*** had eczema or any kind of skin allergy? NO ☐ YES ☐

IF 'NO' TO Q28 GO TO Q 29, IF 'YES':

28.1 How old were you when you first had eczema or skin allergy? YEARS ☐☐

28.2 Did/does your eczema or skin allergy affect your hands? NO ☐ YES ☐

28.3 Have you noticed that contact with certain materials, chemicals or anything else **<u>in your work</u>** makes your eczema worse? NO ☐ YES ☐ DON'T KNOW ☐

29. Have you ***ever*** had an itchy rash that was coming and going for at ***least 6 months***? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 30, IF 'YES': NO ☐ YES ☐

29.1 Have you had this itchy rash ***in the last 12 months?***

IF 'NO' GO TO QUESTION 30, IF 'YES':

29.1.1. Has this itchy rash ***at any time*** affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles under the buttocks or around the neck, ears or eyes NO ☐ YES ☐

29.1.2 Has this itchy rash affected your hands at any time <u>in the last 12 months</u>? NO ☐ YES ☐

30. How many times a week did you eat fish <u>when you were a child around age 10 years</u>? **TICK ONE BOX ONLY**

Never	1	☐
Rarely	2	☐
Once a week	3	☐
Several times a week	4	☐
Almost daily	5	☐

31. How many times a week did you eat seafood when you were a child around age 10 years? **TICK ONE BOX ONLY**

Never	1	☐
Rarely	2	☐
Once a week	3	☐
Several times a week	4	☐
Almost daily	5	☐

32. We would like to ask about your parents and grandparents, whether they were ever treated for tuberculosis and when they were born. If you do not know the year of birth, please suggest crudely (nearest 10 years):

	Ever treated for tuberculosis	**Year of birth**	**Main occupation before age 40 years**
Mother	☐ No ☐ Yes ☐ Don't know		
Father	☐ No ☐ Yes ☐ Don't know		
Maternal grandmother	☐ No ☐ Yes ☐ Don't know		
Maternal grandfather	☐ No ☐ Yes ☐ Don't know		
Paternal grandmother	☐ No ☐ Yes ☐ Don't know		
Paternal grandfather	☐ No ☐ Yes ☐ Don't know		

You took part in the last survey in **[month]** in **[year]**. At that time you described your job as **['current' job from last occupational matrix]**

33. I would like to ask you to list all jobs that you have had since the last survey. I am interested in each one of the jobs that you have done for three months or more. These jobs may be outside the house or at home, **excluding homemaking or housework**, full time or part time, paid or unpaid, including self employment, for example in a family business. Please include part time jobs only if you had been doing them for 20 or more hours per week. Please start with your current or last held job.

Job	Occupation – Job Title: *Please provide a detailed description of the job*	Industry / Branch: *What does (did) your firm or employer make or what services does (did) it provide?*	Start month		Start year				End month		End year *(If current job please enter CURRENT)*			
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														

IF JOBS ARE GIVEN GO TO QUESTION 33.1; IF NO JOBS GIVEN GO TO Q34

	NO	YES
33.1 Have you had to change or leave <u>any</u> of these jobs because it affected your breathing?	☐	☐

IF 'NO' GO TO QUESTION 34; IF 'YES':

33.1.1-10 Please indicate which job(s) you had to change or leave (use numbers from question 33).

	NO	YES
33.1.1 Job 1	☐	☐
33.1.2 Job 2	☐	☐
33.1.3 Job 3	☐	☐
33.1.4 Job 4	☐	☐
33.1.5 Job 5	☐	☐
33.1.6 Job 6	☐	☐
33.1.7 Job 7	☐	☐
33.1.8 Job 8	☐	☐
33.1.9 Job 9	☐	☐
33.1.10 Job 10	☐	☐

34. What best describes your current main activity?

	TICK ONE BOX ONLY	
Employed (including employed by temping agencies)	1	☐
Self-employed (entrepreneur, freelance or other)	2	☐
Full time student	3	☐
Full time housewife/househusband	4	☐
Unemployed looking for work	5	☐
Unemployed not looking for work	6	☐
Retired	7	☐
Other	8	☐

IF NOT 'EMPLOYED' OR 'SELF-EMPLOYED' GO TO QUESTION 34.1
IF 'EMPLOYED' OR SELF-EMPLOYED' GO TO QUESTION 34.2;

NO YES

34.1 Were you forced to give up working all together because of asthma, wheezing shortness of breath or other respiratory or lung problems? ☐ ☐

IF 'NO' GO TO QUESTION 35, IF 'YES':

MONTH YEAR

34.1.1 When did this occur? ☐☐ ☐☐☐☐

NOW GO TO QUESTION 35

NO YES

34.2 In your current job, are you regularly exposed to vapours, gas, dust or fumes? ☐ ☐

NO YES

34.3 Does being at your current workplace ever cause breathing problems (chest tightness,wheezing, coughing)? ☐ ☐

IF 'NO' GO TO QUESTION 34.4 , IF 'YES':

34.3.1-5 Can you indicate what gives you breathing problems in your current workplace?

NO YES

34.3.1 Physical exertion ☐ ☐
34.3.2 Exposure to mist, hot or cold temperature ☐ ☐
34.3.3 Exposure to vapours gas dust or fumes ☐ ☐
34.3.4 Other peoples cigarette smoke ☐ ☐
34.3.5 Stress ☐ ☐

NO YES

34.3.6 Do these breathing problems diminish or stop during the weekend or during holidays? ☐ ☐

NO YES

34.4. Within the last 12 months have there been wet or damp spots on surfaces in the room where you usually work (for example on walls, wall paper, ceilings or carpets)? ☐ ☐

NO YES

34.5. Within the last 12 months has there been mould or mildew on any surfaces in the room where you usually work? ☐ ☐

NO YES

34.6. At any time in the last 12 months have you noticed the odour of mould or mildew (not from food) in the room where you usually work? ☐ ☐

NO YES

34.7. Do you regularly use cleaning products or disinfectants in your current job? ☐ ☐

IF 'NO' GO TO QUESTION 35, IF 'YES':

34.7.1-13 In the last 12 months, on how many days a week have you used the following products at work? (SHOW CARD WITH FOLLOWING OPTIONS)

1. Never
2. <1 day/week
3. 1-3 days/week
4. 4-7 days/week

CODE
Enter code 1-4 for all boxes

34.7.1 Bleach

34.7.2 Ammonia

34.7.3 Stain removers or other solvents

34.7.4 Acids (including decalcifiers, liquid scale removers, vinegar, hydrochloric acid, …)

34.7.5 Floor polish or floor wax

34.7.6 Liquid or solid furniture polish or wax

34.7.7 Furniture sprays (atomisers or aerosols)

34.7.8 Sprays for mopping the floor

34.7.9 Glass cleaning sprays (atomisers or aerosols)

34.7.10 Degreasing sprays including oven cleaning sprays (atomisers or aerosols)

34.7.11 (Ethyl) alcohol

34.7.12 Soaps or foams or any other chemical product for disinfecting hands

34.7.13 Any other chemical disinfectant (for example, glutaraldehyde, formaldehyde, chloramine-T, quaternary ammonium compounds)

35. How often do you usually exercise so much that you get out of breath or sweat? TICK ONE BOX ONLY

every day	1
4-6 times a week	2
2-3 times a week	3
once a week	4
once a month	5
less than once a month	6
never	7

36. How many hours a week do you usually exercise so much that you get out of breath or sweat? TICK ONE BOX ONLY

none	1
about ½ hr	2
about 1 hour	3
about 2-3 hours	4
about 4-6 hours	5
7 hours or more	6

	NO	YES
37. Do you avoid taking vigorous exercise because of breathing problems?		

YEAR

38. When was your present home built? | | | | |

YEARS

39. How many years have you lived in your current home? | | |

40. Which best describes the building in which you live? TICK ONE BOX ONLY

a) a one family house detached from any other house?	2
b) a one family house attached to one or more houses?	3
c) a building for two families?	4
d) a building for three or four families?	5
e) a building for five or more families?	6
f) other: _____	8

NB THERE IS NO CODE 1 and NO CODE 7

NUMBER

41. How many rooms does your home have? (exclude kitchen, bathroom, toilet, laundry) ☐☐

NUMBER

42. How many people live in your home? ☐☐

43. Does your home have any of the following?	NO	YES
43.1 central heating	☐	☐
43.2 ducted air heating (forced air heating)	☐	☐
43.3 air conditioning	☐	☐

44. Which of the following appliances do you use for heating or for hot water?	NO	YES
44.1 open coal, coke or wood fire	☐	☐
44.2 open gas fire	☐	☐
44.3 electric heater	☐	☐
44.4 paraffin heater	☐	☐
44.5 gas-fired boiler(located inside the home)	☐	☐
44.6 oil-fired boiler	☐	☐
44.7 portable gas heater	☐	☐
44.8 gas fired boiler (located outside the home eg: balcony)	☐	☐
44.9 fully enclosed wood/coal burning stove	☐	☐
44.10 other: _____	☐	☐

45. What kind of stove do you ***mostly*** use for cooking?	TICK ONE BOX ONLY
a) coal, coke or wood (solid fuel)	1 ☐
b) gas (gas from the mains)	2 ☐
c) electric	3 ☐
d) paraffin (kerosene)	4 ☐
e) microwave	5 ☐
f) gas (gas from bottles or other non-mains source)	6 ☐
g) other: _____	7 ☐

45.1 IF YOU USE GAS FOR COOKING: Which of the following do you have?	NO	YES
45.1.1 gas hob (the area on top for heating for example saucepans)	☐	☐
45.1 2.gas oven (the enclosed area used, for example, for baking or for roasting)	☐	☐

MINUTES

46. ***On average*** how long have you spent cooking with your cooker (hob or oven) ***<u>each day</u>*** over ***<u>the last four weeks</u>***? ☐☐☐

47. ***Over the last four weeks*** when you were cooking did you have a door or window to the outside air open	TICK ONE BOX ONLY
a) most of the time	1 ☐
b) some of the time	2 ☐
c) rarely (or only occasionally)	3 ☐
d) I do not have a door or window that opens to the outside in my kitchen	4 ☐
e) never	5 ☐

48. Do you have an extractor fan over the cooker?	NO	YES	DON'T KNOW
	☐	☐	☐

***IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 49, IF 'YES'*:**

48.1 When cooking, do you use the fan	TICK ONE BOX ONLY
a) all of the time?	1 ☐
b) some of the time?	2 ☐
c) none of the time?	3 ☐

48.2 Does the fan take the fumes outside the house?	NO	YES	DON'T KNOW
	☐	☐	☐

49. Has there been any water damage to the building or its contents, for example, from broken pipes, leaks or floods? NO ☐ YES ☐ DON'T KNOW ☐

***IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 50, IF 'YES'*:**
49.1 Has there been any water damage in the last 12 months? NO ☐ YES ☐ DON'T KNOW ☐

50. ***Within the last 12 months*** have you had wet or damp spots on surfaces inside your home other than in the basement (for example on walls, wall paper, ceilings or carpets)? NO ☐ YES ☐

51. Has there ever been any mould or mildew on any surface, other than food, inside the home? NO ☐ YES ☐ DON'T KNOW ☐

IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 52, IF 'YES'

51.1 Has there been mould or mildew on any surfaces inside the home in the last ***12 months***? NO ☐ YES ☐

***IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 52, IF 'YES'*:**

51.1.1-6 Which rooms have been affected? NO ☐ YES ☐
- 51.1.1 bathroom(s)
- 51.1.2 bedroom(s)
- 51.1.3 living area(s)
- 51.1.4 kitchen
- 51.1.5 basement or attic
- 51.1.6 other: _____

52. Have you noticed the odour of mould or mildew (not from food) in your home at any time in the last 12 months? NO ☐ YES ☐

53. Does the room which you use most at home during the day NO ☐ YES ☐
- 58.1 have fitted carpets covering the whole floor?
- 58.2 contain rugs?
- 58.3 have double glazing/triple glazing?
- 58.4 have visible wet or damp spots?
- 58.5 have an airbrick or open chimney?

54. On what floor is the room which you use most at home during the day? FLOOR ☐☐
(Basement = 00 ,Ground floor=1, First floor=2, Second floor=3 etc)

55. Does your bedroom NO ☐ YES ☐
- 55.1 have fitted carpets covering the whole floor?
- 55.2 contain rugs?
- 55.3 have double glazing/triple glazing
- 55.4 have visible wet or damp spots
- 55.5 have an airbrick or open chimney
- 55.6 have radiators that are the main source of room heating
- 55.7 get condensation on the window especially in the winter
- 55.8 have windows that face the road

56. On what floor is the room in which you sleep? FLOOR
(Basement = 00 ,Ground floor=1, First floor=2, Second floor=3 etc)

57. How old is the mattress you currently sleep on? YEARS

58. Do you sleep with the windows open at night during winter? NO YES

IF 'NO' GO TO QUESTION 59, IF 'YES':

58.1 Do you sleep with the windows open TICK ONE BOX ONLY
- a) all of the time? 1
- b) sometimes? 2
- c) only occasionally? 3

59. Do you keep a cat? NO YES

IF 'NO' GO TO QUESTION 60, IF 'YES' NO YES

59.1 Is your cat (are your cats) allowed inside the house?

59.2 Is your cat (are your cats) allowed in the bedroom?

60. Do you keep a dog? NO YES

IF 'NO' GO TO QUESTION 61, IF 'YES': NO YES

60.1 Is your dog (are your dogs) allowed inside the house?

60.2 Is your dog (are your dogs) allowed in your bedroom?

61. Do you keep any birds? NO YES

IF 'NO' GO TO QUESTION 62, IF 'YES': NO YES

61.1 Are any of these birds kept inside the house?

62. In the last 12 months, how often have you done any of the cleaning in your own home? TICK ONE BOX ONLY
- a) Never 1
- b) On less than 1 day per week 2
- c) On 1 to 3 days per week 3
- d) On 4 to 7 days per week 4

IF 'NEVER' GO TO 63, IF 'EVER':

62.1 In the last 12 months, on how many days a week have you personally used the following cleaning products in your own home? (SHOW CARD WITH FOLLOWING OPTIONS)

1. Never
2. <1 day/week
3. 1-3 days/week
4. 4-7 days/week

CODE
Enter code 1-4 for all boxes

- 62.1.1 Bleach *(NOT bleach used for laundry)*
- 62.1.2 Ammonia
- 62.1.3 Stain removers or other solvents
- 62.1.4 Acids (including decalcifiers, liquid scale removers, vinegar, hydrochloric acid, …)
- 62.1.5 Floor polish or floor wax
- 62.1.6 Liquid or solid furniture polish or wax
- 62.1.7 Furniture sprays (atomisers or aerosols)

62.1.8 Sprays for mopping the floor

62.1.9 Glass cleaning sprays (atomisers or aerosols)

62.1.10 Degreasing sprays including oven cleaning sprays (atomisers or aerosols)

Enter code 1-4

63. How often do you use air freshening sprays (atomisers or aerosols) **yourself** inside your home?

1. Never
2. <1 day/week
3. 1-3 days/week
4. 4-7 days/week

64. We would like to know where you have lived since January 2010

Please give the address, including postcode, of all homes you have lived in **for at least one year since 1990, starting with your current address**

House number	Street name	City	Postcode	Moved in	Lived there until (YEAR)
					current

All centres please note: this information will be used for your centre staff to geocode residence. Please do not attempt to send these data to the coordinating centre.

The variables needed within each centre ultimately will be (rh=residential history)

Rh64.1.1 CURRENT Year moved in
Rh64.1.2 CURRENT Year moved out
Rh64.1.3 CURRENT Geocode
Rh64.2.1 HOUSE 1 Year moved in
Rh64.2.2 HOUSE 1 Year moved out
Rh64.2.3 HOUSE 1 Geocode

NUMBER

65. How many days per week do you commute to work

IF '0' GO TO QUESTION 66; IF ONE OR MORE DAYS

MINUTES

65.1 On average, how much time do you spend travelling to and from work each day (total for both directions)?

65.2 What is your main method of commuting? TICK ONE BOX ONLY

a) Walking or cycling	1	☐
b) In a private car	2	☐
c) Bus	3	☐
d) Train	4	☐
e) Other	5	☐

66. When you are near animals, such as cats, dogs or horses, do you ***ever***	NO	YES
66.1 start to cough?	☐	☐
66.2 start to wheeze?	☐	☐
66.3 get a feeling of tightness in your chest?	☐	☐
66.4 start to feel short of breath?	☐	☐
66.5 get a runny or stuffy nose or start to sneeze?	☐	☐
66.6 get itchy or watering eyes?	☐	☐

IF NO TO ALL SYMPTOMS GO TO QUESTION 67; IF YES TO ONE OR MORE SYMPTOMS

66.7.1-4 Do you have such symptom/s when you are near	NO	YES
66.7.1 cat?	☐	☐
66.7.2 dog?	☐	☐
66.7.3 horse?	☐	☐
66.7.4 other?	☐	☐

67. When you are in a dusty part of the house, or near pillows or duvets do you ***ever***	NO	YES
67.1 start to cough?	☐	☐
67.2 start to wheeze?	☐	☐
67.3 get a feeling of tightness in your chest?	☐	☐
67.4 start to feel short of breath?	☐	☐
67.5 get a runny or stuffy nose or start to sneeze?	☐	☐
67.6 get itchy or watering eyes?	☐	☐

68. When you are near trees, grass or flowers, or when there is a lot of pollen about, do you ***ever***	NO	YES
68.1 start to cough?	☐	☐
68.2 start to wheeze?	☐	☐
68.3 get a feeling of tightness in your chest?	☐	☐
68.4 start to feel short of breath?	☐	☐
68.5 get a runny or stuffy nose or start to sneeze?	☐	☐
68.6 get itchy or watering eyes?	☐	☐

IF 'YES' TO ANY OF THE ABOVE:

68.7.1-4 Which time of year does this happen?	NO	YES
68.7.1 winter	☐	☐
68.7.2 spring	☐	☐
68.7.3 summer	☐	☐
68.7.4 autumn	☐	☐

69. Have you ever had an illness or trouble caused by eating a ***particular*** food or foods?	NO	YES
	☐	☐

IF 'NO' GO TO QUESTION 70, IF 'YES':

69.1 Have you nearly always had the same illness or trouble after eating this type of food?	NO	YES
	☐	☐

IF 'NO' GO TO QUESTION 70, IF 'YES':

69.2 Was this food any of the following?

	NO	YES
69.2.1 Cow's milk*		
69.2.2 Hen's eggs		
69.2.3. Fish		
69.2.4 Shrimp or Lobster		
69.2.5 Peanut		
69.2.6 Hazelnut		
69.2.7 Walnut		
69.2.8 Peach		
69.2.9 Apple		
69.2.10 Kiwi fruit		
69.2.11 Bananas		
69.2.12 Melon		
69.2.13. Tomato		
69.2.14 Celery		
69.2.15 Carrot		
69.2.16 Soybean		
69.2.17 Lentils		
69.2.18 Wheat**		
69.2.19 Buckwheat		
69.2.20 Corn		
69.2.21 Rice		
69.2.22 Sesame seed		
69.2.23 Mustard seed		
69.2.24 Sunflower seed		
69.2.25 Poppy seed		

* Including other cow's milk products such as butter, cheese, yoghurt, crème fraiche, fromage frais….

** Including wheat products such as bread and breakfast cereals

69. 3 Have you had any problems eating any other food or foods? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 69.4, IF 'YES PLEASE LIST THESE FOODS:

69.3.1 Food 1_____ CODE ☐☐☐

69.3.2 Food 2_____ CODE ☐☐☐

CODE

69.4 Please answer each of these questions for the two foods causing the main problems. Please identify the food from the list of foods given (q69.2.1-25). If more than three foods are given in the list provide information on foods in 69.3.1-3. Please list in order of the most severe reaction

FOOD ONE

CODE

69.4.1 Please confirm the name of this food _____ | | | |

69.4.2-11 Did this illness or trouble include	NO	YES
69.4.2 a rash or itchy skin?		
69.4.3 diarrhoea or vomiting?		
69.4.4 runny or stuffy nose?		
69.4.5 severe headaches?		
69.4.6 breathlessness?		
69.4.7 itching, tingling or swelling in the mouth, lips or throat?		
69.4.8 difficulty swallowing?		
69.4.9 fainting or dizziness?		
69.4.10 symptoms so severe you had an emergency injection from a doctor, or had to use an epipen		
69.4.11 other_____		

69.4.12 . How soon after eating this food did you get the first symptoms? TICK ONE BOX ONLY

a) Less than half an hour	1
b) Half an hour to one hour	2
c) One hour to two hours	3
d) Two hours to four hours	4
e) More than four hours	5

YEARS

69.4.13 How old were you when you first had this attack? | | |

YEARS

69.4.14 How old were you when you last had this attack? | | |

NUMBER

69.4.15 How many times has this occurred during your life? | | |

FOOD TWO

CODE

69.5.1 Please confirm the name of this food _____ | | | |

69.5.2-11 Did this illness or trouble include	NO	YES
69.5.2 a rash or itchy skin?		
69.5.3 diarrhoea or vomiting?		
69.5.4 runny or stuffy nose?		
69.5.5 severe headaches?		
69.5.6 breathlessness?		
69.5.7 itching, tingling or swelling in the mouth, lips or throat?		
69.5.8 difficulty swallowing?		
69.5.9 fainting or dizziness?		
69.5.10 symptoms so severe you had an emergency injection from a doctor, or had to use an epipen		
69.5.11 other_____		

69.5.12 . How soon after eating this food did you get the first symptoms? TICK ONE BOX ONLY

a) Less than half an hour	1
b) Half and hour to one hour	2

c) One hour to two hours 3 ☐
d) Two hours to four hours 4 ☐
e) More than four hours 5 ☐

69.5.13 How old were you when you first had this attack? YEARS ☐☐

69.5.14 How old were you when you last had this attack? YEARS ☐☐

69.5.15 How many times has this occurred during your life? NUMBER ☐☐

70. Have you ever smoked for as long as a year? NO ☐ YES ☐

['YES' means at least 20 packs of cigarettes or 12 oz (360 grams) of tobacco in a lifetime, or at least one cigarette per day or one cigar a week for one year]

IF 'NO' GO TO QUESTION 71, IF 'YES':

70.1 How old were you when you started smoking? YEARS ☐☐

70.2 How old were you when you started smoking daily? YEARS ☐☐

Never smoked daily please enter 88

70.3 Do you **now** smoke, as of ***one month ago***? NO ☐ YES ☐

***IF 'NO' GO TO QUESTION 70.4, IF 'YES'*:**

70.3.1-4 How much do you ***now*** smoke on average? NUMBER

70.3.1 number of cigarettes per day ☐☐
70.3.2 number of cigarillos per day ☐☐
70.3.3 number of cigars a week ☐☐
70.3.4 pipe tobacco in a) ounces / week ☐☐
b) grams / week ☐☐☐

70.4 Have you stopped or cut down smoking? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 71, IF 'YES':

70.4.1 Did you stop or cut down due to breathing problems? NO ☐ YES ☐

70.4.2 How old were you when you stopped or cut down smoking? YEARS ☐☐

70.4.3.1-4 ***On average*** of the entire time you smoked, before you stopped or cut down, how much did you smoke? NUMBER

70.4.3.1 number of cigarettes per day ☐☐
70.4.3.2 number of cigarillos per day ☐☐
70.4.3.3 number of cigars a week ☐☐
70.4.3.4 pipe tobacco in a) ounces / week ☐☐
b) grams / week ☐☐☐

71. Have you been **regularly** exposed to tobacco smoke in the last ***12 months***? *['Regularly' means on most days or nights]* NO ☐ YES ☐

IF 'NO' GO TO QUESTION 72, IF 'YES':

71.1. Not counting yourself, how many people in your household smoke regularly? NUMBER ☐☐

71.2 Do people smoke regularly in the room where you work? NO ☐ YES ☐

71.3 How many hours per day are you exposed to ***other people's*** tobacco smoke? HOURS

71.4 How many hours per day, are you exposed to other peoples tobacco smoke in the following locations? HOURS

- at home
- at workplace
- in bars, restaurants, cinemas or similar social settings
- elsewhere

72. Have you used any **inhaled** medicines to help your breathing at any time in the last ***12 months***? NO YES

IF NO' GO TO QUESTION 73, IF 'YES':

Which of the following have you used in the last ***12 months***?

NO YES

72.1 <u>short acting</u> beta-2-agonist (only) inhalers

(Please include combinations that include beta 2 and steroids in section 72.6)

72.1.1 If used, which one? _____

72.1.2 What type of inhaler do you use? _____

72.1.3. In the last 3 months, how have you used them: TICK ONE BOX ONLY

- a) when needed 1
- b) in short courses 2
- c) continuously 3
- d) not at all 4

If answer to 72.1.3 is <u>when needed</u>: NUMBER

72.1.4 Number of puffs per month

If answer to 72.1.3 is <u>in short courses</u> NUMBER

72.1.5 number of courses

72.1.6 number of puffs per day

72.1.7 average number of days per month

If answer to 72.1.3 <u>is continuously</u> NUMBER

72.1.8 number of puffs per day

NO YES

72.2 <u>long acting</u> beta-2-agonist inhalers

(Please include combinations that include long acting beta 2 and steroids in section 72.6)

72.2.1 If used, which one? _____

72.2.2 What type of inhaler do you use? _____

72.2.3 In the last 3 months, how have you used them: TICK ONE BOX ONLY

- a) when needed 1
- b) in short courses 2
- c) continuously 3
- d) not at all 4

NO YES

72.3 long acting anti-muscarinic inhalers ☐ ☐

72.3.1 If used, which one? _____ ☐☐

72.3.2 What type of inhaler do you use? _____ ☐

72.3.3 In the last 3 months, how have you used them: TICK ONE BOX ONLY

a) when needed 1 ☐

b) in short courses 2 ☐

c) continuously 3 ☐

d) not at all 4 ☐

NO YES

72.4 long acting anti-muscarinic inhalers and beta-2-agonists (combination therapy) ☐ ☐

72.4.1 If used, which one? _____ ☐☐

72.4.2 What type of inhaler do you use? _____ ☐

72.4.3 In the last 3 months, how have you used them: TICK ONE BOX ONLY

a) when needed 1 ☐

b) in short courses 2 ☐

c) continuously 3 ☐

d) not at all 4 ☐

NO YES

72.5 inhaled steroids (ONLY) ☐ ☐

(Please include combinations that include beta 2 and steroids in section 72.6)

72.5.1 If used, which one? _____ ☐☐

72.5.2 What type of inhaler do you use? _____ ☐

NUMBER

72.5.3. What is the dose per puff (in micrograms)? ☐☐☐☐

72.5.4. In the last 3 months, how have you used them: TICK ONE BOX ONLY

a) when needed 1 ☐

b) in short courses 2 ☐

c) continuously 3 ☐

d) not at all 4 ☐

If answer to 72.5.4 is when needed: NUMBER

72.5.5 Number of puffs per month ☐☐

If answer to 72.5.4 is in short courses NUMBER

72.5.6 number of courses ☐☐

72.5.7 number of puffs per day ☐☐

72.5.8 average number of days per month ☐☐

If answer to 72.5.4 is continuously NUMBER

72.5.9 number of puffs per day ☐☐

NUMBER

72.5.10 How many times over the last 3 months have you temporarily increased this treatment because your symptoms became worse? ☐☐

NO YES

72.6 inhaled steroids and beta2 agonists <u>(combined therapy)</u> ☐ ☐

72.6.1 If used, which one? _____ ☐☐

72.6.2 What type of inhaler do you use? _____ ☐

NUMBER

72.6.3. What is the dose per puff (in micrograms)? ☐☐☐☐

(Please insert the dose of the inhaled steroid)

72.6.4. In the last 3 months, how have you used them: TICK ONE BOX ONLY

a) when needed 1 ☐

b) in short courses 2 ☐

c) continuously 3 ☐

d) not at all 4 ☐

If answer to 72.6.4 is <u>when needed</u>: NUMBER

72.6.5 Number of puffs per month ☐☐

If answer to 72.6.4 is in <u>short courses</u> NUMBER

72.6.6 number of courses ☐☐

72.6.7 number of puffs per day ☐☐

72.6.8 average number of days per month ☐☐

If answer to 72.6.4 is <u>continuously</u> NUMBER

72.6.9 number of puffs per day ☐☐

NUMBER

72.6.10 How many times over the last 3 months have you temporarily increased this treatment because your symptoms became worse? ☐☐

NO YES

72.7 inhaled steroids, <u>long acting</u> anti-muscarinic inhalers and beta-2-agonists (triple therapy) ☐ ☐

72.7.1 If used, which one? _____ ☐☐

72.7.2 What type of inhaler do you use? _____

NUMBER

72.7.3 What is the dose per puff (in micrograms)? ☐☐

(Please insert the dose of the inhaled steroid)

72.7.4 In the last 3 months, how have you used them: TICK ONE BOX ONLY

a) when needed 1 ☐

b) in short courses 2 ☐

c) continuously 3 ☐

d) not at all 4 ☐

If answer to 72.7.4 is <u>when needed</u>: NUMBER

72.7.5 Number of puffs per month ☐☐

If answer to 72.7.4 is in <u>short courses</u> NUMBER

72.7.6 number of courses ☐☐

72.7.7 number of puffs per day ☐☐

72.7.8 average number of days per month ☐☐

If answer to 72.7.4 is <u>continuously</u> NUMBER

72.7.9 number of puffs per day ☐☐

NUMBER

72.7.10 How many times over the last 3 months have you temporarily ☐☐

increased this treatment because your symptoms became worse?

73. Have you used any **pills**, **capsules**, **tablets** or **medicines**, other than inhaled medicines, to help your breathing at any time in the last ***12 months***? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 74, IF 'YES':
Which of the following have you used in the last ***12 months***?

73.1 **oral methylxanthines** NO ☐ YES ☐

73.1.1 if used, which one? _____ ☐☐
73.1.2 what dose of tablet ☐☐☐

73.1.3. In the last 3 months, how have you used them: TICK ONE BOX ONLY
a) when needed 1 ☐
b) in short courses 2 ☐
c) continuously 3 ☐
d) not at all 4 ☐

73.2 **oral steroids** NO ☐ YES ☐

73.2.1 If used, which one? _____ ☐☐
73.2.2 what dose of tablet ☐☐☐

73.2.3. In the last 12 months, how have you used them: TICK ONE BOX ONLY
a) when needed 1 ☐
b) in short courses 2 ☐
c) continuously 3 ☐

If answer to 73.2.3 is <u>when needed</u>: NUMBER
73.2.4 number of tablets per month ☐☐

If answer to 73.2.3 is in <u>short courses</u> NUMBER
73.2.5 number of courses ☐☐
73.2.6 tablets per day ☐☐
73.2.7 average number of days per month ☐☐

If answer to 73.2.3 is <u>continuously</u> NUMBER
73.2.8 tablets per day ☐☐

73.2.9. Have you used them in the last ***3 months***? NO ☐ YES ☐

73.3 oral anti-leukotrienes NO ☐ YES ☐

73.3.1 If used, which one? _____ ☐☐
73.3.2 what dose of tablet ☐☐☐

73.3.3. In the last 3 months, how have you used them: TICK ONE BOX ONLY
a) when needed 1 ☐

b) in short courses 2 ☐
c) continuously 3 ☐
d) not at all 4 ☐

If answer to 73.3.3 is <u>continuously:</u> NUMBER

73.3.4 Number of tablets per day ☐☐

NO YES

74. Have you **<u>ever</u>** used inhaled steroids (show list, including combined therapy)? ☐ ☐

IF NO GO TO QUESTION 75; IF YES

NO YES

74.3. Have you used inhaled steroids ***every year*** since the last survey? ☐ ☐

IF 'NO' GO TO QUESTION 74.4: IF 'YES' MONTHS

74.3.1 On average how many months each year have you taken them? ☐☐

NOW GO TO QUESTION 75

YEARS

74.4 How many of the years since the last survey have you taken inhaled steroids? ☐☐

IF 'NONE' ENTER 00 AND GO TO QUESTION 75; IF 'YES' MONTHS

74.4.1 On average how many months of each of these years have you taken them? ☐☐

NO YES

75. Have you had a course of antibiotics in the last 12 months to help your breathing? ☐ ☐

IF NO GO TO QUESTION 76;IF YES NUMBER

75.1 How many courses of antibiotics? ☐☐

NO YES

76. Have you used antibiotics for nasal/sinus problems in the last 12 months? ☐ ☐

NO YES

77. Have you **<u>ever</u>** had any vaccinations or injections for the treatment of allergy or had a course of desensitisation? ☐ ☐

IF NO GO TO QUESTION 78; IF YES CODE

77.1 What was this treatment?_____ ☐☐

NO YES

77.2 Have you had this treatment in the last 12 months? ☐ ☐

IF HAS HAD ANOTHER VACCINATION, INJECTION OF DESENSITISATION CODE

77.3 What was this treatment?_____ ☐☐

NO YES

77.4 Have you had this treatment in the last 12 months? ☐ ☐

NO YES

78. Have you **<u>ever</u>** had any injections for the treatment of asthma? ☐ ☐

IF NO GO TO QUESTION 79; IF YES CODE

78.1 What was this treatment? (Include biologics in the code list) _____ ☐☐

NO YES

78.2 Have you had this treatment in the last 12 months? ☐ ☐

NO YES

79. Have you **<u>ever</u>** had any injections for the treatment of atopic dermatitis, nasal polyps or chronic rhinosinusitis? ☐ ☐

IF NO GO TO QUESTION 80;IF YES CODE

79.1 What was this treatment? (Include biologics in the code list)_____ ☐☐

NO YES

79.2 Have you had this treatment in the last 12 months? ☐ ☐

NO YES

80. Are you usually vaccinated against flu? ☐ ☐

IF NO GO TO QUESTION 81;IF YES NO YES

80.1 Were you vaccinated against flu in the last winter period? ☐ ☐

81. Have you been vaccinated against pneumonia (Pneumovax) in the last 5 years? NO ☐ YES ☐ DON'T KNOW ☐

82. Have you been vaccinated against covid-19? NO ☐ YES ☐ DON'T KNOW ☐

IF NO GO TO QUESTION 83;IF YES

82.1 Which vaccination were you given?_____ CODE ☐☐

82.2 When did you receive your first dose? Month Year ☐☐

FOR 2 DOSE VACCINES

82.3 When did you receive THE SECOND DOSE? Month Year ☐☐

83. Has your doctor ever prescribed medicines, including inhalers, for your breathing? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 84, IF 'YES':

83.1 If you are prescribed medicines for your breathing, do you ***normally*** take

TICK ONE BOX ONLY

a) all of the medicine? 1 ☐
b) most of the medicine? 2 ☐
c) some of the medicine? 3 ☐
d) none of the medicine? 4 ☐

83.2 ***When your breathing gets worse***, and you are prescribed medicines for your breathing, do you normally take

TICK ONE BOX ONLY

a) all of the medicine? 1 ☐
b) most of the medicine? 2 ☐
c) some of the medicine? 3 ☐
d) none of the medicine? 4 ☐

83.3 Do you think it is bad for you to take medicines all the time to help your breathing? NO ☐ YES ☐

83.4 Do you think you should take as much medicine as you need to get rid of ***all*** your breathing problems? NO ☐ YES ☐

84. What medication, regardless of cause, have you taken regularly for more than 6 of the last 12 months? *(DO NOT include the respiratory medication given in previous questions)*

IF NONE, PROCEED TO Q85, OR COMPLETE THE TABLE

	Medication (name)	A	N	N	A	A	N	N
84.1								
84.2								
84.3								
84.5								
84.6								
84.7								
84.8								
84.9								
84.10								

A=letter N=digit (of seven alphanumeric ATC code)

85. Do you have or have you ever had any of the following illnesses. If yes, please indicate the age you were first diagnosed with the disease?

		NO	YES				YEARS	
85.1.1	Stroke				85.1.2	Age diagnosed		
85.2.1	Angina, heart attack, coronary heart disease				85.2.2	Age diagnosed		
85.3.1	Insulin dependent diabetes				85.3.2	Age diagnosed		
85.4.1	Non-insulin dependent diabetes				85.4.2	Age diagnosed		
85.5.1	Cancer				85.5.2	Age diagnosed		
85.6.1	Depression				85.6.2	Age diagnosed		
85.7.1	Hypertension				85.7.2	Age diagnosed		
85.8.1	Osteoporosis				85.8.2	Age diagnosed		
85.9.1	Crohns Disease				85.9.2	Age diagnosed		
85.10.1	Migraine				85.10.2	Age diagnosed		
85.11.1	Rheumatoid arthritis				85.11.2	Age diagnosed		
85.12.1	Ankylosing spondylitis, psoariatic arthritis				85.12.2	Age diagnosed		
85.13.1	Gastro-oesophagel reflux hiatus hernia or oesophagitis				85.13.2	Age diagnosed		

85.5.3	Type of cancer	

Code for 85.5.3
1= breast
2= prostate
3= lung

5= other

86. Do you have any long term limiting illness not mentioned above and not including asthma, COPD, chronic bronchitis or emphysema? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 87, IF 'YES':

86.1 Please name this condition_____ CODE ☐☐

87. ***Since the last survey***, have you visited a hospital casualty department or emergency room (for any reason, apart from accidents and injuries)? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 88, IF 'YES':

87.1. Was this due at least once to ***breathing problems***? NO ☐ YES ☐

87.2 Have you visited a hospital casualty department or emergency room (for any reason, apart from accidents and injuries) **in the last 12 months**? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 88, IF 'YES':

87.2.1 How many times in the last ***12 months***? TIMES ☐☐

87.2.2 Among these ones, how many times because of ***breathing problems***? TIMES

[Write '0' if s/he had not visited the emergency room for breathing problems] ☐☐

88. ***Since the last survey***, have you spent a night in hospital (for any reason, apart from accidents and injuries)? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 89, IF 'YES':

88.1 Was this due at least once to ***breathing problems***? NO ☐ YES ☐

88.2 Have you spent a night in hospital (for any reason, apart from accidents and injuries) **in the last 12 months**? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 89, IF 'YES':

88.2.1 How many nights in the last ***12 months***? NIGHTS ☐☐

88.2.2 Was this due at least once to ***breathing problems***? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 89, IF 'YES':

88.3 In the last ***12 months*** how many nights have you been hospitalized in each of the following types of ward for ***breathing problems***?

88.3.1 intensive care unit NIGHTS ☐☐

88.3.2 other ☐☐

89. In the last ***12 months*** have you been seen by a general practitioner (for any reason, apart from accidents and injuries)? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 90, IF 'YES':

89.1 How many times **in the last 12 months**? TIMES ☐☐

89.2 Of these, how many were for ***breathing problems***? TIMES ☐☐

[Write '0' if not been seen by general practitioner in the last 12 months for breathing problems]

90. In the last ***12 months*** have you seen a specialist (for any reason, apart from accidents and injuries)? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 91, IF 'YES':

90.1 How many times in the last ***12 months***? TIMES ☐☐

90.2 How many times have you seen a specialist (chest physician, allergy specialist, internal medicine specialist, ENT doctor) because of ***breathing problems in the last 12 months***? TIMES ☐☐

[Write '0' if not been seen by a specialist in the last 12 months for breathing problems]

91. In the last ***12 months*** have you had any clinical or laboratory tests because of health problems (apart from accidents and injuries)? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 92, IF 'YES':

91.1 Was this due at least once to ***breathing problems***? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 92, IF 'YES':

91.1.1-5 In the last ***12 months*** how many times have you had the following tests for ***breathing problems***?

TIMES

91.1.1 breathing test in a laboratory specially for lung function measures ☐☐

91.1.2 skin test for allergy ☐☐

91.1.3 blood test for allergy ☐☐

91.1.4 x-rays ☐☐

91.1.5 thorax CT ☐☐

92. In the last ***12 months*** have you lost days of work because of health problems (apart from accidents and injuries)? NO ☐ YES ☐ HAVE NOT WORKED IN THE LAST 12 MONTHS ☐

IF NOT WORKED OR HAS NOT LOST DAYS OF WORK GO TO QUESTION 93; IF 'YES':

92.1 How many days **in the last 12 months?** DAYS ☐☐☐

92.2 Among these ones, how many because of breathing problems? ☐☐☐

[Write '000' if not lost any days due to breathing problems]

93. **Since the last survey** were you forced to give up working altogether because of health problems (apart from accidents and injuries)? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 94, IF 'YES':

93.1 When did this occur? MONTH ☐☐ YEAR ☐☐☐☐

93.2 Were you forced to give up working altogether because of **breathing problems**? NO ☐ YES ☐

94. In the last ***12 months*** have there been any days when you have had to ***give up activities other than work*** (e.g. looking after children, the house, studying) because of health problems (apart from accidents and injuries)? NO ☐ YES ☐

IF 'NO' YOU HAVE FINISHED THE QUESTIONNAIRE, IF 'YES':

94.1 How many days **on average** each month? DAYS ☐☐

94.2 Among these ones, how many because of ***breathing problems***? DAYS ☐☐

[Write '0' if s/he has not had any days of activity lost due to breathing problems]

95. Interview type TICK ONE BOX ONLY

1 face to face interview at clinic ☐
2 telephone ☐
3 face to face at home ☐
4 other ☐

96. Date of birth check. What is the date of birth of this participant? DAY ☐☐ MONTH ☐☐ YEAR ☐☐☐☐

97. Which of the following best describes you? TICK ONE BOX ONLY

1 Single ☐
2 Married/cohabiting ☐
3 Separated/Divorced ☐
4 Widowed ☐
5 Other or do not wish to answer ☐

Appendix B. Sun exposure questionnaire

Centre					
ID					

Your exposure to sunlight

Please complete the following questionnaire

"Working days" are those days you are at work (normally weekdays but if you work during the weekends, you should consider them as a working day).

"Non-working days" are those days that you are off work that can be either weekends or weekdays that you are away from work but not on holiday.

"Holidays" are extended leisure period (at least four days) away from work devoted to rest or pleasure.

1. In the last 12 months, from April to September, how many hours per day (on average) did you spend outdoors in the daytime on the following occasions -

		HOURS
1.1	Working days	
1.2	Non-working days	
1.3	Holidays	

For example if you spend 2 hours outdoors you need to write 02

2. In the last 12 months, from October to March, how many hours per day (on average) did you spend outdoors in the daytime on the following occasions-

		HOURS
2.1	Working days	
2.2	Non-working days	
2.3	Holidays	

For example if you spend 2 hours outdoors you need to write 02

3. In the last 12 months, how many days did you spend in each of following destinations for your summer holidays? DAYS

3.1	Country of residence	
3.2	Northern European (Nordic countries, Estonia, Northern Russia (Moscow, St Petersburg and above)	
3.3	**Southern European countries** (Albania, Bosnia and Herzogovina, Bulgaria, Cyprus, Southern Italy (below Rome), Greece, Southern France (below Lyon), Macedonia, Malta, Montenegro, Portugal, Spain, Turkey	
3.4	**Other European** (Not mentioned above)	
3.5	**Other Non-European** (Please give country name_____	
	Country code for 3.5	

(Write 00 for those destinations that you have not been to)

4. During **summer days**, which parts of your body are **usually** exposed to sunlight when you are outside **on working days?** **(Tick appropriate boxes)**

		NO	YES
4.1	Face and hands		
4.2	Shoulders		
4.3	Arms		
4.4	Legs		
4.5	Trunk		

5. During **summer days**, which parts of your body are **usually** exposed to sunlight when you are outside **on non-working days?** **(Tick appropriate boxes)**

		NO	YES
5.1	Face and hands		
5.2	Shoulders		
5.3	Arms		
5.4	Legs		
5.5	Trunk		

6. During **summer days**, which parts of your body are **usually** exposed to sunlight when you are outside **on holidays?** **(Tick appropriate boxes)**

		NO	YES
6.1	Face and hands		
6.2	Shoulders		
6.3	Arms		
6.4	Legs		
6.5	Trunk		

7. During **summer days** how often do you use sunscreen when you are outside **on the following occasions**

(Tick appropriate box)		**NEVER**	**SOME-TIMES**	**USUALLY**	**ALWAYS**
7.1	Working days				
7.2	Non-working days				
7.3	Holidays				

8. When applying sunscreen, which **SPF** Factor do you usually use? **FACTOR** | | | *(If more than one, the one that you use the most)* *(Write 00, if you don't use sunscreen)*

9. What is your skin type?

Colour	**Characteristic** (Tick appropriate box)		
White – typically Celtic	Always burns, never tans but freckles		1
White	Usually burns, light tan with difficulty		2
White	Sometimes burns, moderate to good tan		3
Olive – typically Mediterranean	Rarely burns, dark tan with ease		4
Brown – e.g. Indian sub-continent	Very rarely burns, tans very easily		5
Black – e.g. of African origin	Never burns, tans very easily		6

TIMES

10. In the last 12 months how many times have you used sun beds solarium)? | | | | (or a

IF YOU <u>HAVE NOT</u> USED SUNBEDS IN THE LAST 12 MONTHS YOU HAVE COMPLETED THE QUESTIONNAIRE

IF YOU <u>HAVE</u> USED SUNBEDS IN THE LAST 12 MONTHS

MINUTES

10.1. On average how long did you stay on the sunbed solarium) each time? | | | (or

<u>Thank you for completing this questionnaire</u>

Appendix C. Measurements of lung function with reversibility

Trained staff should carry out each spirometry session according to the SOP described in the Section below.

During a spirometry manoeuvre there is a small risk that the participant may faint and hurt him/herself while falling. **Participants must therefore perform the manoeuvres in the seated position, in a chair with arms but without wheels.**

Spirometry will be conducted using the ndd EasyOne Spirometer. This is a highly portable spirometer that measures flow and volume by ultra-sound transit time. It is endorsed by the ERS and complies with ATS spirometry standards.

To ensure data integrity equipment must be regularly cleaned and the calibration checked daily according to manufacturer instructions. **Always check that the EasyOne configuration settings are set to the study parameters and install the Easy Ware software in the English language version.**

During each session the following measures will be collected:

Forced Vital Capacity (FVC)	The total volume of air exhaled in a forced expiratory manoeuvre.
Forced Expiratory Volume at One Second (FEV_1)	The amount of air that a person exhales during the first second of a forced expiratory manoeuvre.
The ratio of FEV_1 to the FVC (FEV_1/FVC)	It is obtained by dividing the FEV_1 by the FVC, and is expressed as a percentage (100 x FEV_1/FVC).
Forced Expiratory Volume at Six Seconds (FEV_6)	The amount of air that a person exhales during the first six seconds of a forced expiratory manoeuvre.
The ratio of FEV_1 to the FEV_6 (FEV_1/FEV_6)	An alternative to the FEV_1/FVC ratio.

These volumes are measured **before and after bronchodilator administration.**

Location

Spirometry testing ideally should be performed in a private, temperature-controlled room. All necessary equipment should be available in the room. Ideally the room should be well lit, preferably with a window, and located in a quiet area of a clinic. For safety, the participant must be seated in a chair with arms but without wheels.

Equipment

The spirometry session should be carried out in a room with the following equipment:

- Sink for hand washing, soap and hand towels

- Containers of:

  - clean mouthpieces (Spirettes)

  - nose-clips
- Containers to collect:

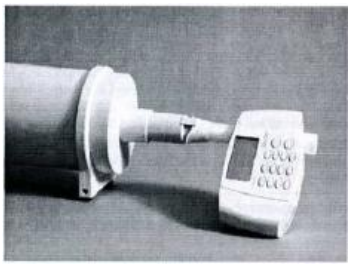
  - used Spirettes

  - used nose clips
- Box of tissues
- Alcohol wipes
- Disposal bin
- Clinical gloves
- Chair with arms/without wheels
- Spare AA batteries
- EasyOne Spirometer
- Calibration syringe & syringe adapter
- Bronchodilator (Ventolin)
- Drinking water and cups/glasses

Calibration

The EasyOne Spirometer has been designed to need no calibration. The instrument can however develop faults and we request that a calibration check be carried out **daily** during the course of the data collection. Instructions for performing the calibration check is in the ndd EasyGuide technical manual.

The calibration syringe and adapter should always be stored next to the spirometer so that the temperature between them is similar. Contact the co-ordinating centre **immediately** if the EasyOne develops a fault.



Medication use prior to testing

In order to provide a valid lung function assessment, participants should be asked to refrain from taking bronchodilators before their clinical visit appointment. The exact omission time depends on the type of medication. The extent to which you are able to ask this of participants may be governed by your local ethics committee

Type of medication	**Avoid for:**
short-acting beta-2 agonist	4 hours prior to the visit
anticholinergic inhaler	4 hours prior to the visit

oral beta-2 agonist	8 hours prior to the visit
oral theophylline	8 hours prior to the visit
oral antimuscarinic	8 hours prior to the visit
long-acting beta-2 agonist (Serevent)	12 hours prior to the visit

If the participant has not been able to comply with these waiting periods, the spirometry can be done anyway, AS LONG AS THEY HAVE NOT TAKEN ANY INHALER IN THE HOUR PRIOR TO TESTING. It is preferable that the participant make another appointment if they are willing.

Participants should also refrain from smoking for one hour prior to testing.

Reasons for rescheduling spirometry testing

In some instances, spirometry testing may be contraindicated by a temporary condition that would affect the validity of the manoeuvre or endanger the health of the participant. These situations are at the discretion of the investigator/ spirometry technician – examples may include: acute back pain; a respiratory tract infection with unresolved symptoms in the week prior to the visit; or recent dental work.

Ideally, centres should postpone testing and should re-schedule the visit for a time when the situation could be expected to be resolved. If participants are brought back later for spirometry testing, but the rest of their data are collected on the first visit, then the Spirometry safety questions must be asked again and the date of spirometry entered onto Questionnaire.

Contraindications for testing

Testing should **not** be done if the subject has or reports any of the following:

- a heart attack in the last three months
- chest or abdominal surgery in the past 3 months
- a detached retina or eye surgery in the past 1 month
- if they are a woman in the last trimester of pregnancy
- any other co-morbidity (such as unstable angina or pneumonia) that, in the opinion of a local clinician, may affect the performance of the test or impact the participant's safety

If a participant has or reports any of the conditions above do not proceed with spirometry. If they agree, participants may be brought back for retesting at a later date.

Method

A detailed description of the use and operation of the ndd EasyOne spirometer, together with instructions for coaching the participant, are included in the ndd EasyGuide users' manual. All study staff who undertake the lung function tests are asked to read this document and to be familiar with its contents and that of this SOP. A copy of this document should be kept with each spirometer in case questions arise during testing.

Always check that the EasyOne configuration settings are set to the study parameters.

A nominated person responsible for configuration of the EasyOne^{TM} should be designated at each clinical site.

Participant information should be entered into the spirometer as prompted. In the ID field enter all digits of the subject's unique ID.

As prompted enter the age, height, weight, ethnic category, gender, smoking status and allocated project staff ID of the person undertaking the test (Always input your same allocated 'Staff ID' -this is your two digit or two figure personal ID or initials, always use the same ID)

If after safety questions it is decided to reschedule the session, ensure that the same questionnaire is recalled for use at the second visit. If testing is to proceed offer participants the opportunity to use toilet facilities before testing. Instruct them to loosen any tight clothing that might restrict inspiration. Testing should be conducted with the participant seated, upright and with chin slightly elevated on a chair with arms but no wheels. The chair is a safety measure to support the participant in case s/he faints during the manoeuvre.

Staff and participants should wash their hands before the start of the test and use a tissue or gloves to remove mouthpieces (the Spirette) from its packaging. Allow the participant to insert the clean Spirette into the spirometer. Be careful to ensure that the arrow on the Spirette is lined up with the arrow on the spirometer.

All manoeuvres should be performed with the participant wearing a nose clip. This clip prevents air from moving through the nose during the test.

A good rapport with the participant will improve the quality of the test. Explain that the purpose of the test is to take some measurements to check on the health of the lungs. Emphasize that, although the procedure does not hurt, in order to get useful and valid results he/she must breathe out as hard and as fast and for as long as is possible when told to do so, and will need to repeat the procedure a few times.

Pre-bronchodilator test

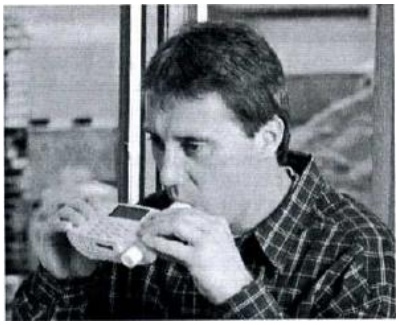
Lung function testing should be carried out AFTER the 'GETTING READY FOR FENO, SPIROMETRY, REVERSIBILITY AND BIOIMPEDENCE QUESTIONNAIRE' has been completed.

After instructing the participant about the procedure for pulmonary function testing the following procedures (outlined in sections 5.2 to 5.4 of the ndd EasyGuide™ users' manual) should be followed. This initial series of manoeuvres is performed **BEFORE** administering the bronchodilator.

Explain that the participant should:

- take in as deep a breath as possible
- when his/her lungs are totally full, quickly position the mouthpiece
- BLAST out the air as hard and as fast as possible
- blow out smoothly **without re-breathing.**
- continue exhaling for at least 6 seconds
- throughout they should remain erect and not bend forward

To assist the participant – technicians should give a vigorous demonstration in which they demonstrate the correct positioning of the mouthpiece take a deep breath and emphasize the full depth of inhalation demonstrate a dramatic blast out as fast as possible.



Follow the instructions in the box regarding number of blows to be conducted.

Baseline spirometry
All participants to have a minimum of **5 attempts** at a full FVC manoeuvre.

Grade A achieved - go on to bronchodilator
Grade B achieved - go on to bronchodilator

If after 5 attempts grade A or grade B **not** achieved continue for 3 further attempts.

As soon as grade A **or** grade B achieved – go on to bronchodilator

If after 8 attempts Grade C achieved – go on to bronchodilator
If after 8 attempts Grade C **not** achieved – go on to bronchodilator

Post-bronchodilator spirometry
All participants to have a minimum **of 3 attempts** at a full FVC manoeuvre post-bronchodilator

Grade A achieved – the test is complete

If after **5 attempts**

Grade A has been achieved – the test is complete
Grade B has been achieved – the test is complete

If after 5 attempts grade A or grade B **not** achieved continue for 3 further attempts

As soon as grade A or grade B achieved – the test is complete

If after 8 attempts grade C is achieved – the test is complete
If after 8 attempts grade C is not achieved – the test is complete

Administer the bronchodilator

Administer **two puffs** of bronchodilator (short-acting beta-agonist, Salbutamol, 100 mcg per puff) to the participant using a standard spacer e.g. Clement Clarke Able Spacer . A new unit should be used for each individual unless appropriate sterilisation procedures are approved by your centre, and used units should be disposed of in the appropriate manner.

The following steps should be followed

- The fieldworker shakes the inhaler and places it on the spacer
- The participant is asked to exhale fully, tip their chin up slightly and place their lips around the spacer.
- The fieldworker discharges the inhaler into the spacer using either the middle or index finger, and holding the spacer level and securely with their thumb beneath
- The participant inhales slowly and deeply to total lung capacity and then hold their breath for 10 seconds
- The procedure is repeated for steps 2-5

For optimal distribution of the bronchodilator, these steps should be followed carefully. A timer should be set up to sound 15 minutes after the last administered puff.

Maximum Post-bronchodilator manoeuvre

The post-bronchodilator (BD) manoeuvre can start anytime **after the 15-minute wait.** It is not critical that the post-BD manoeuvre be done immediately at 15 minutes, but rather that it is done at least 15 minutes after the last administered puff of bronchodilator.

Problems with lung function testing

Many factors will result in error, including hesitation or false starts, cough, variable effort, glottis closure, early termination and leaks. When errors do occur, review them with the participant before proceeding with additional manoeuvres. You may wish to repeat a demonstration manoeuvre. Demonstrate the correct placement of the mouthpiece, emphasize the maximum depth of inhalation, and then blast out the air. If the participant tries again and the reproducibility criteria are not met, continue the test as needed (up to a total of 8 manoeuvres), assuming that the participant is able to continue.

When errors occur, review common errors with the participant before proceeding with additional manoeuvres.

Ask the participant to watch the technician perform the FVC manoeuvre again. The technician should demonstrate the correct placement of the mouthpiece, emphasize the maximum depth of inhalation, and then blast out the air. If the participant tries again and the reproducibility criteria are not met, the technician should continue administering the test as needed (up to a total of five manoeuvres), assuming that the subject is able to continue.

Some participants may never be able to provide three reproducible manoeuvres. The goal of each session is to meet the acceptability and reproducibility criteria, but these are not absolute requirements for data to be used.

Spirometer calibration, maintenance and hygiene

The EasyOne spirometer is designed to reduce the need for cleaning and maintenance (see sections 13 and 14 in the EasyGuide users' manual). The surface of the spirometer and cradle may be cleaned by wiping with a damp cloth. If a more thorough cleaning is desired, the spirometer and its spirette cavity may be cleaned with an alcohol wipe or a soft

cloth that has been lightly moistened with isopropyl alcohol. **Do not let liquids flow into the Spirette cavity of the spirometer while cleaning.** The disposable Spirette eliminates the need for cleaning the spirometer between patients. The Spirettes are designed for single patient use only, and must be removed and disposed of after each participant. Nose clips should be thoroughly cleaned after each use with hot water and detergent, allowed to dry and then wiped with alcohol.

Participants with evidence of obvious upper respiratory infections should not be tested, but rather asked if they may be tested at a later date.

Beyond battery replacement and the calibration check, the spirometer requires no maintenance. No service should be performed on the spirometer except by manufacturer-authorised personnel.

Data transfer

Centres will be required to have ndd EasyWare PC-software which is compatible with a PC running Microsoft Windows 98/ME/2000/XP. EasyWare software is available in a number of languages, however centres are asked to **install the software in the English language version**. This is important. All databases will be regularly merged with the master database at the co-ordinating centre.

Data should be transferred to a local PC daily. From here they will be transferred to the co-ordinating centre.

Quality Control Checks

Data management including quality control measures for all lung function measurements will be performed centrally headed by researchers with extensive experience from former ECHRS and Constances study waves. We will apply quality control measures aligned with earlier study waves.

At various points during the study the coordinating centres will request spirometric data from each centres so that the Spirometry Curves arising from the testing each technician has done can be reviewed. Explicit instruction will be provided to each centre at the time for the transfer of pseudo-anonymised data and a brief report will be provided to each centre.

Versions of NDD software
All centres should use the SAME software throughout the period of the study – centres should NOT upgrade during the period of data collection.

Centres buying new NDD will be working with firmware that may be version 6.2 upwards. This is satisfactory

Centres using NDD that have already been purchased should upgrade their machine prior to starting the study to version 5.8.

EasyOne configuration settings

Test settings:

Parameter	
Predicted:	ERS/ECCS
Add.Ped:	'blank'
Value Sel:	Best Value
Interpretation:	OFF or 'blank'
Lung Age:	OFF
Automated QC:	ON
FVC Selection:	FVC
PEF Unit:	L/s
AfricanEthnCorr:	88%
AsianEthnCorr:	100%
HispanicEthnCorr:	100%
OtherEthnCorr:	100%
Storage:	3 Best Curves or 'all curves'

General Settings:

Parameter	
Time Form:	24 hour
Date Form:	DD/MM/YY
Date:	Enter date
Time:	Enter local time
Alpha-ID:	No
Tech.ID:	Yes
SyringeVol:	3.0L
Height Unit:	m/cm
Weight Unit:	Kg
Age/Birth:	Age
LCDContrast:	40% or adjust as needed
Language:	English
Altitude:	0 (or nearest 500meters)
Mode	DIAGNOSTIC
Temperature	°C
Humidity	Best average guess

Report Settings:

Parameter	
Printer:	Set to printer type used
Data:	3 Best Data or 3 Best Values
Curve:	3 Best or 3 best curves
Graph:	Small FV & VT
Headers (1-4)	Enter the headers you want

Appendix D. Measurement of height, weight, waist/hip circumference and bioimpedance

Height, weight and bioimpedence should be made after completion of the 'Getting ready for FENO, spirometry, reversibility and bioimpedence' questionnaire.
Height and weight must be measured before spirometry. Even if spirometry is not going to be done these measures should be made

Height

Height is a predictor of lung function and it is very important that this is measured correctly by trained staff. No matter how simple the equipment, staff should be trained to record height and weight according to the guidelines in section below.

Height should be recorded to the nearest complete 1 cm using the same stadiometer for all measurements.

The Harpenden wall mounted or pocket Stadiometer is recommended. Stadiometers attached to balance beam scales are not recommended.The type of stadiometer used should be provided in the Centre Equipment Inventory that is completed when data are forwarded to the coordinating centre

1. Ask the participant to remove shoes, hat and bulky clothing such as coats and sweaters. You may need to ask some participants to adjust hairstyles or remove hair accessories that may interfere with measurement.

2. The participant should stand erect, with shoulders level, hands at sides, knees or thighs together and with weight evenly distributed on both feet. Feet should be flat on the floor (or foot piece) with both heels comfortably together and touching the base of the vertical board or wall. When possible, all four contact points (the head, back, buttocks, and heels) should touch the vertical surface while the participant also maintains a natural stance. Some people may not be able to keep a natural stance if all four contact points were touching the vertical surface. For these participants, at a minimum, two contact points — the head and buttocks, or the buttocks and heels — should always touch the vertical surface.

3. Ask the participant to move their head or position the participant's head by placing a hand on the chin and moving it into the Frankfort Plane. The Frankfort Plane is an imaginary line from the lower margin of the eye socket to the notch above the tragus of the ear. When aligned correctly, the Frankfort Plane is parallel to the horizontal headpiece and perpendicular to the vertical back piece of the stadiometer. This is best viewed and aligned when the investigator is directly to the side and at eye level with the participant.

4. Lower the horizontal headpiece until it firmly touches the crown of the head and is at a right angle with the measurement surface. Ask the subject to inhale deeply and check contact points to

ensure that the lower body stays in the proper position and heels remain flat. Reposition the head board if necessary. Read the height to the nearest complete 1 cm. If the reading is to xxx.5cm always round down to the nearest complete 1cm. Do not round up. Record results immediately and enter into this value into the spirometer when prompted.

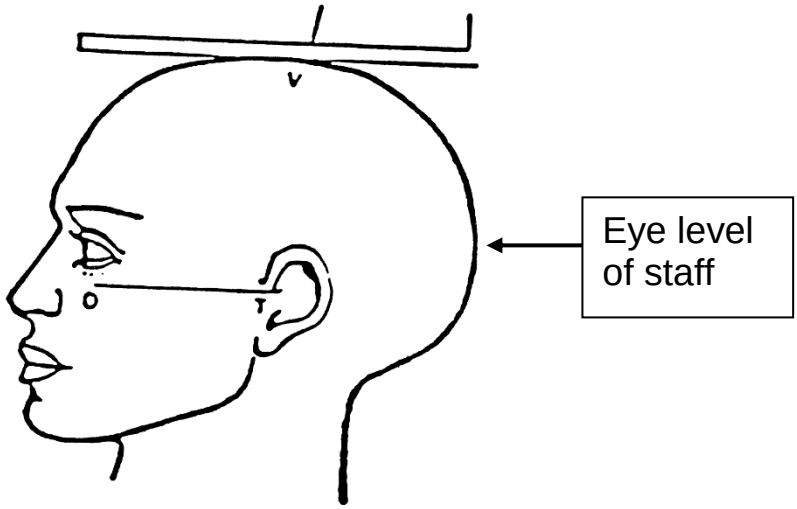


Figure 1 Frankfort Plane for measuring body height

Weight

Weight should be measured to the nearest to 1 kg. Weight is not used as a predictor of lung function, but accuracy is still important and staff should be trained to use centres' weighing equipment correctly. The measurements should be recorded to the nearest 1kg.

A digital scale or balance beam is recommended for the measurement of weight. The same scale should be used for all measurements. Ideally the scales should be calibrated at least annually by a local procedure.

Whatever kind of scale is to be used, checks should be made and any necessary adjustments to ensure that the scale reads '0' before each measurement.

The scales should be placed on a flat, firm floor surface. If weight has to be measured in carpeted areas, a small sheet of wood or hard plastic should be placed beneath the scale. The participant should ideally be wearing normal lightweight indoor clothing. Ask them to remove shoes, coats, jacket and heavy objects from pockets such as telephones or keys. Ask the participant to step onto the centre of the scale platform and stand up straight with arms relaxed at their sides and looking straight forward.

Staff training for height and weight measures

Staff involved in the recruitment should be properly trained to conduct height and weight measurements based on the on the method described here. Training should begin with a discussion and demonstration of the methods. The 'trainee' should then be asked to perform duplicate measurements on three different individuals. Height and weight should be recorded for each individual once and then the process repeated for a second recording of measurement. The 'trainer' should also undertake the same measurements on one occasion. Adequate training is achieved where the trainee's repeat measurements are within 1kg and 1cm of each other and the mean of the repeat measurements are within 1kg and 1cm of the trainer's measurements. If reproducibility is not met, repeat the training process -beginning with a review of the methods, until the required standards are achieved.

Waist and hip circumferences

Measurement should be made with an insertion tape calibrated in mm, with a plastic or metal buckle at one end.

All measurements should be taken to the nearest millimetre, and are recorded on the forms as centimetres with one decimal place.

Before starting measurements ask the participant to 1) remove all outer layers of clothing (eg: jackets, heavy or baggy jumpers, cardigans and waistcoats) 2) remove shoes with heels, 3) remove tight garments intended to alter the shape of the body (eg corsets, lycra body suits, support tights) and 4) remove or loosen belts.

Ensure the respondent is standing erect in a relaxed manner and breathing normally. Weight should be evenly balanced on both feet and the feet should be about 25-30cm (1 foot) apart. The arms should be hanging loosely at their sides. If possible, kneel or sit on a chair to the side of the respondent. Pass the tape around the body of the respondent and insert the plain end of the tape through the metal ring at the other end of the tape. To check the tape is horizontal you have to position the tape on the right flank and peer round the participant's back from his/her left flank to check that it is level. This will be easier if you are kneeling or sitting on a chair to the side of the respondent. Hold the buckle flat against the body and flatten the end of the tape to read the measurement from the outer edge of the buckle. Do not pull the tape towards you, as this will lift away from the respondent's body, affecting the measurement.

Measuring waist circumference

1. The waist is defined as the point midway between the iliac crest and the costal margin (lower rib). To locate the levels of the costal margin and the iliac crest use the fingers of the right hand held straight and pointing in front of the participant to slide upward over the iliac crest. Men's waists tend to be above the top of their trousers whereas women's waists are often under the waistband of their trousers or skirts.

2. Do not try to avoid the effects of waistbands by measuring the circumference at a different position or by lifting or lowering clothing items. For example, if the respondent has a waistband at the correct level of the waist (midway between the lower rib margin and the iliac crest) measure the waist circumference around the waistband.

3. Ensure the tape is horizontal. Ask the participant to breathe out gently and to look straight ahead (to prevent the respondent from contracting their muscles or holding their breath). Take the measurement at the end of a normal expiration. Measure to the nearest millimetre and record this on the schedule.

4. Repeat this measurement again.

5. If your second waist measurement differs by 3cm or more from the first please check and repeat the measure.

6. If you are of the opinion that clothing, posture or any other factor is significantly affecting the waist measurement, record this on the schedule.

Measuring hip circumference

1. The hip circumference is defined as being the widest circumference over the buttocks and below the iliac crest. To obtain an accurate measurement you should measure the circumference at several positions and record the widest circumference.

2. Check the tape is horizontal and the respondent is not contracting the gluteal muscles. Pull the tape, allowing it to maintain its position but not to cause indentation. Measure to the nearest millimetre and record this on the schedule.

3. If clothing is significantly affecting the measurement, record this on the schedule.

4. Repeat this measurement again.

5. If your second hip measurement differs by 3cm or more from the first please check and repeat the measure.

General points

The tape should be tight enough so that it doesn't slip but not tight enough to indent clothing. If clothing is baggy, it should be folded before the measure is taken.

If the respondent is large, ask him/her to pass the tape around rather than having to "hug" them. Remember though to check that the tape is correctly placed for the measurement being taken and that the tape is horizontal all the way around.

If you have problems palpating the rib, ask the respondent to breathe in very deeply. Locate the rib and as the respondent breathes out, follow the rib as it moves down with your finger. If your respondent has a bow at the back of her skirt, this should be untied as it may add a substantial amount to the waist circumference. Female respondents wearing jeans may present a problem if the waistband of the jeans is on the waist at the back but dips down at the front. It is essential that the waist measurement is taken midway between the iliac crest and the lower rib and that the tape is horizontal. Therefore in this circumstance the waist measurement would be taken on the waist band at the back and off the waist band at the front. Only if the waistband is over the waist all the way around can the measurement be taken on the waistband. If there are belt loops, the tape should be threaded through these so they don't add to the measurement.

We only want to record problems that will affect the measurement by more than would be expected when measuring over light clothing. As a rough guide only record a problem if you feel it affected the measurements by more than 0.5cm. We particularly want to know if waist and hip are affected differently.

Bioimpedence

Bioelectric impedence should be measured using a suitable instrument that delivers a 50KHZ current and which provides a direct measure of reactance and resistance (not derived values for impedence or fat free mass).

Recommended equipment is new version of the BodyStat 1500 MDD (**NOT** the BodyStat 1500). Each unit has a serial number which can be displayed by holding down the down arrow key whilst switching the unit on at the same time. If the serial number starts 301 then it is the older device and will not display Resistance or Reactance. If the serial number starts 310 then it is the newer device and will display Resistance and Reactance. (NB the BodyStat 1500 is NOT suitable as it does not display reactance or resistance)

The following participants should not have their bioimpedence measured:

- Women who are pregnant
- Those who have a pacemaker or defibrillator
- Those who have cardiac failure, renal disease or liver disease such that they have visible oedema of the legs, or ascites.

Participants should refrain from drinking in the hour prior to measurement.

Participants should:

- Remove all metal jewellery from their body and any metal objects from their pockets.
- Remove their right shoe and any socks or stockings on the right foot
- Lie on their back on a non-conductive surface (examination table, bench, carpet)
- Relax and lay their head back
- Place their feet 20 to 25 centimetres apart, ensuring the upper inner thighs are not touching
- Place their hands 10 centimetres or more from their sides so that the inner upper arm is not touching their torso

The fieldworker should now place sensor pads on the participant's right hand and right foot.

The sensor pads on the hand are placed midway along an imaginary line running from the head of the ulna to the head of the radius with one half of the pad above the line and one half below the line and with the tab facing way from the body and about 1cm above the knuckle line towards the middle of the hand with the tab facing way from the body

The sensor pads on the foot are placed midway along an imaginary line over the crest of the ankle and connecting the lateral and medial malleoli with one half of the pad above the line and one half below the line and with the tab facing way from the body about 1cm above the toe line towards the middle of the foot and with the tab facing way from the body.

The fieldworker should check that the electrodes are properly adhered to the participants skin with at least 75% of the pad in contact with the skin.

Measures will be made at 50 KHZ.

Reactance and resistance at 50KHZ should be recorded.

Two readings should be made, checking the positioning of all electrodes and the position of the participant prior to the second reading

Phase angle, total body water, fat mass and fat free mass will be calculated as derived variables using available relevant formulae available at the time of the analysis.

Scheme for data on height, weight and waist and hip circumferences

DATA FOR HEIGHT,WEIGHT, WAIST-HIP AND BIOIMPEDENCE

Centre | | | |

ID | | | | | |

HEIGHT, WEIGHT, WAIST-HIP

3.	Height		cm
4.	Weight		kg
5.1	Waist 1		cm
5.2	Waist 2		cm
6.1	Hip 1		cm
6.2	Hip 2		cm

		NO	YES
7	Problem with waist hip measures		

7.1 Describe _____

BIOIMPEDENCE MEASURE 1

8 Resistance | | | | ohms

9 Reactance | | • | | ohms

BIOIMPEDENCE MEASURE 2

10 Resistance | | | | ohms

11 Reactance | | • | | ohms

Appendix E. Food frequency questionnaire (FFQ)

This questionnaire asks for background information related to what you eat. We would like you to describe the frequency of consumption in the last 12 months of the foods listed.

Your answers will be treated as strictly confidential and will be used only for the purposes of this research.

Please fill in the following boxes:

Date today	Date of birth	Indicate whether a
DD/MM/YYYY	DD/MM/YYYY	Female (F) or Male (M)

Dear Participant:

In the context of the ECRHS IV/EPHOR-study, we would like to ask you to complete and return this food frequency questionnaire (FFQ). Please tick (√) in the box to indicate how often, on average, you have eaten the specified amount of each food during the last 12 months. Do not tick more than one box per food.

Because this FFQ is being used in several countries, YOU WILL BE UNFAMILIAR WITH some of the foods listed in this questionnaire. If you do not eat some of these, please tick the option "Rarely/never".

If you make a mistake and put a tick in the wrong box just cross through the tick as shown below, and put a tick in the correct box.

EXAMPLE

Vegetables excluding potatoes (medium serving)	Rarely/ Never	1-3 times a month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 day	4+ day
Lettuce			√	√ (crossed)				

PLEASE TICK **ONE BOX ONLY** PER LINE AND DO NOT LEAVE FOODS WITHOUT ANSWER.

For seasonal fruits such as strawberries or grapes, if you eat them about once a week when in season, you should put a tick in the column "once a week".

We thank you very much for your collaboration.

ECRHS IV Team

Tick one box for every food to show how often you ate it. Please answer every question, if you are uncertain about how to answer a question then do best you can, but please do not leave a question blank.

Bread and rolls **(one slice or medium serving)**	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q1p1 Total bread (any, on average)	1	2	3	4	5	6	7	8
q1p2 Wholemeal or brown bread (with or without seeds)	1	2	3	4	5	6	7	8
q1p3 White bread (e.g. baguette, rolls, sliced crust(less))	1	2	3	4	5	6	7	8
q1p4 Rye bread (any)	1	2	3	4	5	6	7	8
q1p5 Nan bread	1	2	3	4	5	6	7	8
q1p6 Chapatti	1	2	3	4	5	6	7	8
q1p7 Yeast based bread	1	2	3	4	5	6	7	8

Breakfast and other cereals (any) **(medium serving)**	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q2p1 Any breakfast cereals (e.g. oatmeal, wheat germ, cornflakes, Quaker, kasha)	1	2	3	4	5	6	7	8
q2p2 wheat germ	1	2	3	4	5	6	7	8
q2p3 Quaker	1	2	3	4	5	6	7	8
q2p4 Corn-flakes	1	2	3	4	5	6	7	8
q2p5 Weetabix	1	2	3	4	5	6	7	8

Semolina **(table spoon)**	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q3p1 Couscous in savoury dishes	1	2	3	4	5	6	7	8
q3p2 Couscous in sweet dishes	1	2	3	4	5	6	7	8

Pasta (and wheat derived foods) (medium serving)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q4p1 Any pasta (on average)	1	2	3	4	5	6	7	8
q4p2 Plain (refined) pasta (e.g. spaghetti, macaroni)	1	2	3	4	5	6	7	8
Pasta (and wheat derived foods) (medium serving) continued)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q4p3 Plain wholemeal (unrefined) pasta	1	2	3	4	5	6	7	8
q4p4 Filled pasta (with meat/cheese/vegetables)	1	2	3	4	5	6	7	8
q4p5 Noodles (excluding rice noodles)	1	2	3	4	5	6	7	8

Bakery products/desserts (one biscuit, one unit, or medium serving)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q5p1 Any cakes or pastries (on average)	1	2	3	4	5	6	7	8
q5p2 Cakes (e.g. sponge, chocolate, ginger, honey, apple)	1	2	3	4	5	6	7	8
q5p3 Pastries (e.g. croissants)	1	2	3	4	5	6	7	8
q5p4 Rolls (with/without stuffing)	1	2	3	4	5	6	7	8
q5p5 Muffins	1	2	3	4	5	6	7	8
q5p6 Doughnuts, buns (plain or filled)	1	2	3	4	5	6	7	8
q5p7 Rice pudding	1	2	3	4	5	6	7	8
q5p8 Cheese cake	1	2	3	4	5	6	7	8
q5p9 Pancakes	1	2	3	4	5	6	7	8
q5p10 Plain biscuits (with no fillings or cream)	1	2	3	4	5	6	7	8
q5p11 Thin biscuits (e.g. crackers, rice-wafer)	1	2	3	4	5	6	7	8

Rice (1 cup (cooked))	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q6p1 Rice (any)	1	2	3	4	5	6	7	8
q6p2 White rice	1	2	3	4	5	6	7	8
q6p3 Brown/wholemeal (unrefined) rice	1	2	3	4	5	6	7	8
q6p4 Rice noodles	1	2	3	4	5	6	7	8

Sugar (tea spoon) & jam (enough for 1 slice of bread)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q7p1 Table sugar	1	2	3	4	5	6	7	8
q7p2 Jam	1	2	3	4	5	6	7	8
q7p3 Marmalade	1	2	3	4	5	6	7	8
q7p4 Honey	1	2	3	4	5	6	7	8
q7p5 Syrup spreads	1	2	3	4	5	6	7	8

Sugar products excluding chocolate (one unit)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q8p1 Total (any) sweets or bonbons	1	2	3	4	5	6	7	8
q8p2 Boiled sweets, toffees, caramels	1	2	3	4	5	6	7	8
q8p3 Mixed candies	1	2	3	4	5	6	7	8
q8p4 Cereal bars, flapjacks/fruit bar	1	2	3	4	5	6	7	8
q8p5 Water ice (lolly ice)	1	2	3	4	5	6	7	8

Chocolate	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q9p1 Chocolates (any)	1	2	3	4	5	6	7	8
q9p2 Chocolate snack bars, e.g. Mars, Crunchie (1 bar)	1	2	3	4	5	6	7	8
q9p3 Chocolate (e.g. plain, dark/milk) (a square or 20g)	1	2	3	4	5	6	7	8

Vegetable oils (1 table spoon)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q10p1 Vegetable oil (blended, any)	1	2	3	4	5	6	7	8
q10p2 Sunflower oil	1	2	3	4	5	6	7	8
q10p3 Olive oil	1	2	3	4	5	6	7	8
q10p4 Extra virgin olive oil	1	2	3	4	5	6	7	8
q10p5 Palm oil	1	2	3	4	5	6	7	8

11. Margarine and lipids of mixed origin (1 table spoon)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q11p1Any margarine or spread (excluding soya spread)	1	2	3	4	5	6	7	8
q11p2 Low-fat margarine	1	2	3	4	5	6	7	8
q11p3 Normal margarine	1	2	3	4	5	6	7	8
q11p4 Blended spreads	1	2	3	4	5	6	7	8
q11p5 Soya-based spreads (any)	1	2	3	4	5	6	7	8
q11p6 Any margarines or vegetable spreads fortified with omega-3	1	2	3	4	5	6	7	8
111p7 Margarines or spreads fortified with vitamin D	1	2	3	4	5	6	7	8

Butter and animal fats (amount spread enough to cover a loaf of bread)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q12p1 Any butter	1	2	3	4	5	6	7	8
q12p2 Low-reduced fat butter	1	2	3	4	5	6	7	8
q12p3 Normal butter	1	2	3	4	5	6	7	8

Nuts (10 units approx.)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q13p1 Any nuts	1	2	3	4	5	6	7	8
q13p2 Peanuts	1	2	3	4	5	6	7	8
q13p3 Cashew nuts	1	2	3	4	5	6	7	8
q13p4 Almonds	1	2	3	4	5	6	7	8
q13p5 Walnuts	1	2	3	4	5	6	7	8

Legumes (1 cup (cooked))	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2+ per day	4+ day
q14p1Any legumes	1	2	3	4	5	6	7	8
q14p2 Kidney (red), black beans	1	2	3	4	5	6	7	8
q14p3 Lentils	1	2	3	4	5	6	7	8
q14p4 Chickpeas (also hummus)	1	2	3	4	5	6	7	8
q14p5 Cluster beans (guar)	1	2	3	4	5	6	7	8
q14p6 French beans (string beans)	1	2	3	4	5	6	7	8
q14p7 Fava beans	1	2	3	4	5	6	7	8
q14p8 Soya beans	1	2	3	4	5	6	7	8

Vegetables excluding potatoes (medium serving)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q15p1 Any vegetables (excluding potatoes)	1	2	3	4	5	6	7	8
q15p2 Lettuce	1	2	3	4	5	6	7	8
q15p3 Spinach (including lamb's quarters)	1	2	3	4	5	6	7	8
q15p4 Chard	1	2	3	4	5	6	7	8
q15p5 Fenugreek	1	2	3	4	5	6	7	8
q15p6 Wild greens (e.g. amaranth, purslane, watercress)	1	2	3	4	5	6	7	8
q15p7 Okra	1	2	3	4	5	6	7	8
q15p8 Tomato	1	2	3	4	5	6	7	8
q15p9 Aubergine	1	2	3	4	5	6	7	8
q15p10 Courgette	1	2	3	4	5	6	7	8
q15p11 Sweet peppers (e.g. red, green, yellow)	1	2	3	4	5	6	7	8
q15p12 Cucumber	1	2	3	4	5	6	7	8
q15p13 Bitter melon (Karela)	1	2	3	4	5	6	7	8
q15p14 Carrots	1	2	3	4	5	6	7	8
q15p15 Parsnip	1	2	3	4	5	6	7	8
q15p16 Turnip or Swede	1	2	3	4	5	6	7	8
q15p17 Artichokes	1	2	3	4	5	6	7	8
q15p18 Radish	1	2	3	4	5	6	7	8
q15p19 Beetroot	1	2	3	4	5	6	7	8
q15p20 Celery	1	2	3	4	5	6	7	8
q15p21 Coleslaw	1	2	3	4	5	6	7	8
q15p22 Sweet Corn	1	2	3	4	5	6	7	8
q15p23 Asparagus	1	2	3	4	5	6	7	8
q15p24 Herbs (e.g. mint, fennel, chive, basil, dill, coriander, parsley) (1 table spoon)	1	2	3	4	5	6	7	8
q15p25 Leek	1	2	3	4	5	6	7	8
q15p26 White/other mushrooms	1	2	3	4	5	6	7	8
q15p27 Onion	1	2	3	4	5	6	7	8
q15p28 Garlic	1	2	3	4	5	6	7	8
q15p29 Cauliflower	1	2	3	4	5	6	7	8
q15p30 Pumpkin	1	2	3	4	5	6	7	8

Vegetables excluding potatoes *(continued)* (medium serving)	Rarely/ Never	1-3 times a month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 day	4+ day
q15p31 Brussels sprouts	1	2	3	4	5	6	7	8
q15p32 Peas (green)	1	2	3	4	5	6	7	8
q15p33 Broccoli	1	2	3	4	5	6	7	8
q15p34 Cabbage (e.g. white, green red, Savoy)	1	2	3	4	5	6	7	8
q15p35 Stuffed vegetables (e.g. vine/green leaves with rice or meat)	1	2	3	4	5	6	7	8
q15p36 Pickled vegetables (e.g. cucumber, radish, cabbage)	1	2	3	4	5	6	7	8
q15p37 Ginger (e.g. in savoury and sweet dishes, in infusion)	1	2	3	4	5	6	7	8

Starchy roots or potatoes (medium serving)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q16p1 Potatoes (on average, in all forms)	1	2	3	4	5	6	7	8
q16p2 Mashed potatoes	1	2	3	4	5	6	7	8
q16p3 Baked/roasted/casserole	1	2	3	4	5	6	7	8
q16p4 Chips/French fries	1	2	3	4	5	6	7	8
q16p5 In salads	1	2	3	4	5	6	7	8
q16p6 Potato dumpling, bread dumpling, gnocchi	1	2	3	4	5	6	7	8
q16p7 Potato tortilla (omelette)	1	2	3	4	5	6	7	8
q16p8 Sweet potato	1	2	3	4	5	6	7	8

Fruits (one piece of fruit)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q17p1 Fresh fruits (any)	1	2	3	4	5	6	7	8
Hard fruits								
q17p2 Apple	1	2	3	4	5	6	7	8
q17p3 Pear	1	2	3	4	5	6	7	8
Stoned fruits								
q17p4 Avocado	1	2	3	4	5	6	7	8
q17p5 Mango	1	2	3	4	5	6	7	8
q17p6 Apricot	1	2	3	4	5	6	7	8

Fruits **(one piece of fruit)**	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
Stoned fruits (continued)								
q17p7 Nectarine	1	2	3	4	5	6	7	8
q17p8 Peach	1	2	3	4	5	6	7	8
q17p9 Plum	1	2	3	4	5	6	7	8
Cherries & Berries								
q17p10 Cherries (1 cup)	1	2	3	4	5	6	7	8
q17p11 Rhubarb	1	2	3	4	5	6	7	8
q17p12 Forest fruits - Berries (e.g. blueberry, strawberry, blackcurrants, blackberry raspberry) (1 cup)	1	2	3	4	5	6	7	8
Soft fruits								
q17p13 Banana	1	2	3	4	5	6	7	8
q17p14 Melon/ Watermelon	1	2	3	4	5	6	7	8
q17p15 Grape (1 cup or 15 grapes)	1	2	3	4	5	6	7	8
q17p16 Squeezed fresh fruit (1 cup)	1	2	3	4	5	6	7	8
q17p17 Pineapple (1/3 of a unit)	1	2	3	4	5	6	7	8
Citrus fruits								
q17p18 Kiwi	1	2	3	4	5	6	7	8
q17p19 Lemon (juice of 1 unit)	1	2	3	4	5	6	7	8
q17p20 Orange	1	2	3	4	5	6	7	8
q17p21 Mandarin/Tangerine	1	2	3	4	5	6	7	8
q17p22 Grapefruit	1	2	3	4	5	6	7	8
Tinned fruits								
q17p23 Tinned fruits (any, 1 can)	1	2	3	4	5	6	7	8
Dried fruits & Olives								
q17p24 Raisin, sultana (1 table spoon)	1	2	3	4	5	6	7	8
q17p25 Fig	1	2	3	4	5	6	7	8
q17p26 Prune	1	2	3	4	5	6	7	8
q17p27 Olives (e.g. black, green) (5 units)	1	2	3	4	5	6	7	8
q17p28 Dates (1 table spoon or 3 units)	1	2	3	4	5	6	7	8

Fruit juices (1 glass 200 ml)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q18p1 Concentrated juice, with sugar	1	2	3	4	5	6	7	8
q18p2 Concentrated juice, without sugar (with sweetener)	1	2	3	4	5	6	7	8

Non-alcoholic beverages (1 glass 200ml)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q19p1 Carbonated/soft/isotonic drinks	1	2	3	4	5	6	7	8
q19p2 Tap water	1	2	3	4	5	6	7	8
q19p3 Mineral water (e.g. still or sparkling)	1	2	3	4	5	6	7	8

Tea/coffee (1 cup)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q20p1 Black tea (any)	1	2	3	4	5	6	7	8
q20p2 Coffee (instant or ground)	1	2	3	4	5	6	7	8
q20p3 Greek (Turkish) Coffee	1	2	3	4	5	6	7	8
q20p4 Green tea	1	2	3	4	5	6	7	8
q20p5 Peppermint tea	1	2	3	4	5	6	7	8
q20p6 Other herbal infusions	1	2	3	4	5	6	7	8

Beer (1/2 pint or 1 glass 200 ml)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q21 Beer (any)	1	2	3	4	5	6	7	8

Wine (1 glass125 ml)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q22p1 Any wine	1	2	3	4	5	6	7	8
q22p2 Red wine	1	2	3	4	5	6	7	8
q22p3 White wine	1	2	3	4	5	6	7	8
q22p4 Rose wine	1	2	3	4	5	6	7	8

23. Other alcoholic beverages (1 glass 50 ml)	Rarely/ Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2+ day	4+ day
q23p1 Fortified wines (Liqueurs) (e.g. Sherry, port, Madeira)	1	2	3	4	5	6	7	8
q23p2 Spirits (e.g. whisky, vodka, rum, gin)	1	2	3	4	5	6	7	8

Red meat and meat products (medium serving)	Rarely Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q24p1 Any red meat (e.g. beef, veal, lamb, pork, game)	1	2	3	4	5	6	7	8
q24p2 Hot/cold roast beef, boiled beef, beef steak, fillet, loin	1	2	3	4	5	6	7	8
q24p3 Beef burger	1	2	3	4	5	6	7	8
q24p4 Minced beef meat (e.g chilli con carne, Bolognese sauce, meatballs)	1	2	3	4	5	6	7	8
q24p5 Meat stew, casserole, in curry	1	2	3	4	5	6	7	8
q24p6 Pork cutlet, chop, steak, fillet, loin, pork ribs	1	2	3	4	5	6	7	8
q24p7 Meat pies	1	2	3	4	5	6	7	8
q24p8 Sausages	1	2	3	4	5	6	7	8
q24p9 Veal	1	2	3	4	5	6	7	8
q24p10 Small game (e.g. rabbit, goat, pheasant, duck)	1	2	3	4	5	6	7	8
q24p11 Other game (e.g. deer, moose)	1	2	3	4	5	6	7	8
q24p12 Lamb (e.g. in stews, kebabs)	1	2	3	4	5	6	7	8
Smoked/cured meat (1 slice)								
q24p13 Cured pork (cold or hot-cooked)	1	2	3	4	5	6	7	8
q24p14 Gammon, ham (e.g. Serrano, prosciutto)	1	2	3	4	5	6	7	8
q24p15 Dried cured sausages (chorizo, salchichon, salami)	1	2	3	4	5	6	7	8
q24p16 Frankfurter	1	2	3	4	5	6	7	8
q24p17 Bacon, bacon cubes	1	2	3	4	5	6	7	8
q24p18 Smoked lamb	1	2	3	4	5	6	7	8
q24p19 Smoked game (any)	1	2	3	4	5	6	7	8

Poultry (medium serving)	Rarely Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q25p1 Any poultry with skin	1	2	3	4	5	6	7	8
q25p2 Any poultry without skin	1	2	3	4	5	6	7	8
Fresh (un-smoked)								
q25p3 Chicken, boiled, roasted, chicken burgers	1	2	3	4	5	6	7	8
q25p4 Chicken in stews, breadcrumbs, pies, fricassee, etc	1	2	3	4	5	6	7	8
q25p5 Turkey, roasted, boiled, strips, etc	1	2	3	4	5	6	7	8
q25p6 Turkey in stews, breadcrumbs, pies, etc.	1	2	3	4	5	6	7	8
Smoked or cured poultry								
q24p7 Any smoked/cured poultry	1	2	3	4	5	6	7	8

Offal (medium serving)	Rarely Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q26p1 Liver (eg panita), pates, potted meat	1	2	3	4	5	6	7	8
q26p2 Other offal (e.g. tongue, brain, heart, kidney, tripe)	1	2	3	4	5	6	7	8

Fish and seafood (medium serving)	Rarely Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q27p1 Any fish (fresh, tinned, smoked, fatty, white)	1	2	3	4	5	6	7	8
q27p2 Fresh fatty fish (e.g. salmon, tuna, trout, anchovy, herring, mackerel, sardine, gravalex, eel)	1	2	3	4	5	6	7	8
q27p3 Fresh white fish (e.g. hake/burbot, cod, haddock, plaice, whiting)	1	2	3	4	5	6	7	8
q27p4 Other fresh fish/seafood products (e.g. taramasalata)	1	2	3	4	5	6	7	8
q27p5 Fresh Crustaceans and molluscs (e.g. mussel, crab, calamari, octopus, cuttlefish, shrimp, clam)	1	2	3	4	5	6	7	8
Fish and seafood (medium serving) (*continued)*	Rarely Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q27p6 Cured or smoked fatty fish (e.g. sardines, tuna, salmon, kipper)	1	2	3	4	5	6	7	8

q27p7 Cured or smoked white fish (e.g. cod, bacalhau)	1	2	3	4	5	6	7	8
q27p8 Tinned fatty fish (e.g. sardines, tuna, salmon)	1	2	3	4	5	6	7	8
q27p9 Tinned crustaceans and molluscs (e.g. mussel, crab, calamari, octopus, cuttlefish, shrimp, clam)	1	2	3	4	5	6	7	8

Eggs (from hen) **(1 egg)**	Rarely Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q28p1 Eggs (any, on average)	1	2	3	4	5	6	7	8
q28p2 Eggs (fried/poached/boiled/hard boiled/in sandwiches)	1	2	3	4	5	6	7	8
q28p3 Egg-based savoury dishes	1	2	3	4	5	6	7	8
q28p4 Egg-based desserts (e.g. Egg cakes, tarts, egg and nuts sweets)	1	2	3	4	5	6	7	8

Milk & dairy (animal and soya) (1 glass/200ml)	Rarely Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q29p1 Milk (any, excluding soya)	1	2	3	4	5	6	7	8
Cow milk								
q29p2 Sour milk (alone/with fruits)	1	2	3	4	5	6	7	8
q29p3 Full-fat milk	1	2	3	4	5	6	7	8
q29p4 Semi-skimmed milk	1	2	3	4	5	6	7	8
q29p5 Skimmed milk	1	2	3	4	5	6	7	8
q29p6 Fermented milk (pro-biotics)	1	2	3	4	5	6	7	8
q29p7 Condensed milk	1	2	3	4	5	6	7	8
Milk (cow) & soya (1 glass/200ml) *(continued)*	Rarely Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
Fortified milks (any animal source)								
q29p8 Vitamin D milk	1	2	3	4	5	6	7	8
q29p9 Omega 3 milk	1	2	3	4	5	6	7	8
Goat or sheep milk								
q29p10 Full fat milk	1	2	3	4	5	6	7	8
q29p11 Semi-skimmed milk	1	2	3	4	5	6	7	8
q29p12 Farmer's milk	1	2	3	4	5	6	7	8

Yoghurt								
q29p13 Yogurt (any type)	1	2	3	4	5	6	7	8
q29p14 Greek-style yoghurt	1	2	3	4	5	6	7	8
q29p15 Fromage frais	1	2	3	4	5	6	7	8
q29p16 Yogurt with probiotics	1	2	3	4	5	6	7	8
Soya based dairy								
q29p17 Soya milk (any)	1	2	3	4	5	6	7	8
q29p18 Soya based yogurt	1	2	3	4	5	6	7	8

Cheeses **(1 regular piece or spread for 1 slice of bread)**	Rarely Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q30p1 Any cheese	1	2	3	4	5	6	7	8
q30p2 Hard cheeses (e.g. Cheddar, parmesan)	1	2	3	4	5	6	7	8
q30p3 Soft cheeses (e.g. Brie, camembert, Philadelphia, tomini, boursault, brinza, chaource, coulommiers, Humboldt fog, kochkase)	1	2	3	4	5	6	7	8
q30p4 Semi-hard cheeses (e.g. Gouda, Emmental/Edam)	1	2	3	4	5	6	7	8
q30p5 Cottage cheese (cheese curd) (natural/with scents)	1	2	3	4	5	6	7	8
q30p6 Hard and semi-hard Greek cheeses (e.g. Kaseri, kefalotiri, Grafiera, Kefalograviera, Ladotiri)	1	2	3	4	5	6	7	8
q30p7 Fresh cheeses (e.g. Feta, mozzarella)	1	2	3	4	5	6	7	8

Other milk derived products **(1 table spoon unless otherwise stated)**	Rarely Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q31p1 Ice cream (1 unit)	1	2	3	4	5	6	7	8
q31p2 Single cream crème	1	2	3	4	5	6	7	8
q31p3 Crème fraîche	1	2	3	4	5	6	7	8
a31p4 Sour cream	1	2	3	4	5	6	7	8
q31p5 Double or clotted cream	1	2	3	4	5	6	7	8
q31p6 Tofu	1	2	3	4	5	6	7	8

Miscellaneous food	Rarely Never	1-3 times a month	Once a week	2-4 week	5-6 week	Once a day	2-3 day	4+ day
q32p1 Dressing sauces (1 table spoon) (e.g. French, Cesar, thousand islands)	1	2	3	4	5	6	7	8
q32p2 Mayonnaise (1 table spoon)	1	2	3	4	5	6	7	8
q32p3 Fresh meat and vegetable soups (regular bowl)	1	2	3	4	5	6	7	8
q32p4 Fresh vegetable or cereal soups (regular bowl) (e.g. green/other cabbage soup, gazpacho, legumes/peas soup, tarhana)	1	2	3	4	5	6	7	8
q32p5 Fresh meat or offal soups (regular bowl)	1	2	3	4	5	6	7	8
q32p6 Fresh fish soups (regular bowl)	1	2	3	4	5	6	7	8
q32p7 Pizza (any) (1 regular slice)	1	2	3	4	5	6	7	8
q32p8 Spicy sauces (1 table spoon)	1	2	3	4	5	6	7	8
q32p9 Ketchup (1 table spoon)	1	2	3	4	5	6	7	8
q32p10 'fried tomato'	1	2	3	4	5	6	7	8
q32p11 Pesto sauce	1	2	3	4	5	6	7	8
q32p12 Traditional dish 3	1	2	3	4	5	6	7	8
q32p13 Traditional dish 4	1	2	3	4	5	6	7	8
q32p14 Traditional dish 5	1	2	3	4	5	6	7	8

Additional questions:

Products for special nutritional use

Do you REGULARLY take any nutritional supplement? e.g. vitamin C, selenium etc?

q33

Yes ☐

No ☐

If you answered yes to question 33, please indicate:

		Times per week dose is taken	
Nutrient supplement (or brand name)	**Dose taken**	**In summer**	**In winter**
q33p1	q33p1dose	q33p1daily	q33p1week
q33p2	q33p2dose	q33p2daily	q33p2week
q33p3	q33p3dose	q33p3daily	q33p3week
q33p4	q33p4dose	q33p4daily	q33p4week
q33p5	q33p5dose	q33p5daily	q33p5week
Vitamin D only			
q33p6 Cod liver pills	q33p6dose	q33p6daily	q33p6week
q33p7 Cod liver oil	q33p7dose	q33p7daily	q33p7week
q33p8 Fish oil capsules	q33p8dose	q33p8daily	q33p8week

34. Are there any other foods you normally eat once or more a week?

q34

Yes ☐

No ☐

If yes, please list below:

Food (if it is a local dish, and you know the main components or ingredients, please name them)	Usual serving size	Number of times eaten per week
q34p1	q34p1size	q34p1times
q34p2	q34p2size	q34p2times
q34p3	q34p3size	q34p3times
q34p4	q34p4size	q34p4times

35. What kind of fat did you most often use for frying, roasting, grilling, etc?

q35 Select one only please:

Butter	1
Lard/dripping	2
Sunflower oil	3
Solid vegetable fat	4
Margarine	5
Olive oil	6
None	0

36. How often do you add salt to food while cooking?

q36

Always	1
Sometimes	2
Rarely	3
Never	0

37. In the last year, on average, how many times a week did you eat a medium serving (unit/glass or cup) of the following food groups?

Food type	**Times/week**
q37p1 Vegetables (excluding potatoes)	q37p1times
q37p2 Potatoes	q37p2times
q37p3 Fruits and fruit products (excluding fruit juice)	q37p3times
q37p4 Fish	q37p4times
q37p5 Fish products	q37p5times
q37p6 Meat, meat products or meat dishes (including bacon, ham and chicken)	q37p6times
q37p7 Milk (skimmed, full fat, any)	q37p7times

38. Are there any foods you do not eat because they cause you allergy or intolerance?

q38

Yes ☐ No ☐

If yes, please name these foods below:

Food not consumed	Reason
q38p1	q38p1reason
q38p2	q38p2reason
q38p3	q38p3reason
q38p4	q38p4reason

Are you currently following a special diet?

Yes ☐ No ☐

If you answered yes, please indicate if you are following one these diets:

	Yes	No
q39p1 Weight loss	1	2
q39p2 Hypertension	1	2
q39p3 Diabetes Mellitus	1	2
q39p4 Coeliac disease	1	2

Please indicate (tick as appropriate) if you suffer any of the following illnesses:

	Yes	No
q40p1 Hypertension	1	2
q40p2 Heart disease (any)	1	2
q40p3 Diabetes Mellitus Type I	1	2
q40p4 Diabetes Mellitus Type II	1	2
q40p5 Obesity/overweight	1	2

Please write your weight (kg) and height (mt)

q41p1 Weight:	**q41p2** Height:

THANK YOU FOR YOUR COOPERATION!

Appendix F. International Physical Activity Questionnaire (IPAQ-7)

(August 2002)

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

☐ No vigorous physical activities → ***Skip to question 3***

How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

☐ No moderate physical activities → ***Skip to question 5***

How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

Appendix G. Sleep questionnaire

Centre | | | | | |
ID | | | | | |

Your sleep

In this questionnaire there are several kinds of sleep related questions.

Some of the questions you answer by marking a box or estimating a time interval.

Some of the questions are answered by circling the alternative which best describes *how often* an event occurs.

1 How much sleep do you estimate that you get on average each night? HOURS | |

2. How often have the following occurred **in the last THREE months:**

The numbers mean
1: Never or almost never
2: Less than once a week
3: once or twice a week
4: 3- 5 nights/days a week
5: Almost every day or night

(Please circle the number that indicates your response)

2.1	that you have difficulty in getting to sleep at night?	1	2	3	4	5
2.2	that you wake up repeatedly during the night?	1	2	3	4	5
2.3	that you wake up too early and have difficulty in getting to sleep again?	1	2	3	4	5
2.4	that you have heartburn or belching when you have gone to bed?	1	2	3	4	5
2.5	that you feel sleepy in the daytime?	1	2	3	4	5
2.6	that you perspire heavily during the night?	1	2	3	4	5

3. Have you ever been told that you snore when you sleep? NO ☐ YES ☐

***IF 'NO' GO TO QUESTION 4, IF 'YES'*:**

	Never	Seldom	Sometimes	Frequently	Every Time
3.1 In the last ***12 months*** have you been told that you stop breathing or have irregular breathing while you are sleeping?	☐	☐	☐	☐	☐
3.2 Have you woken up all of a sudden with a choking sensation or not being able to breathe in the last ***12 months***?	☐	☐	☐	☐	☐
3.3 Have you been told that you snore loudly or that your snoring disturbs other people in the last ***12 months***?	☐	☐	☐	☐	☐

4. Have you ever been told by doctor that you have sleep apnoea? NO ☐ YES ☐

IF "YES":

If you are currently treated for sleep apnoea what treatment do you have?

a. CPAP
b. oral appliance (bite split)
c. Previous surgery in the throat or nose
d. Other

5. How likely are you to doze off or fall asleep in the following situations in contrast to feeling just tired? This refers to your usual way of life in recent times. Even if you have not done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the most appropriate number for each situation:

0 = would **never** doze
1 = **slight** chance of dozing
2 = **moderate** chance of dozing
3 = **high** chance of dozing

(Enter the number that indicates your answer)

	Situation	**Chance of dozing**
5.1	Sitting and reading	
5.2	Watching TV	
5.3	Sitting inactive in a public place (e.g. a theatre or a meeting)	
5.4	As a passenger in a car for an hour without a break	
5.5	Lying down to rest in the afternoon when circumstances permit	
5.6	Sitting and talking to someone	
5.7	Sitting quietly after a lunch without alcohol	
5.8	In a car, while stopped for a few minutes in the traffic	

Appendix H. 36-Item Short Form Survey Instrument (SF-36)

Questionnaire given below is version 1 (v1). EPHOR/ECRHSIV will use version 2(V2)

RAND CORPORATION HEALTH

36-Item Short Form Survey Instrument (SF-36)

RAND 36-Item Health Survey 1.0 Questionnaire Items

Choose one option for each questionnaire item.

1. In general, would you say your health is:

- ○ 1 - Excellent
- ○ 2 - Very good
- ○ 3 - Good
- ○ 4 - Fair
- ○ 5 - Poor

2. **Compared to one year ago**, how would you rate your health in general **now**?

- ○ 1 - Much better now than one year ago
- ○ 2 - Somewhat better now than one year ago
- ○ 3 - About the same
- ○ 4 - Somewhat worse now than one year ago
- ○ 5 - Much worse now than one year ago

The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
3. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	○ 1	○ 2	○ 3
4. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	○ 1	○ 2	○ 3
5. Lifting or carrying groceries	○ 1	○ 2	○ 3
6. Climbing **several** flights of stairs	○ 1	○ 2	○ 3
7. Climbing **one** flight of stairs	○ 1	○ 2	○ 3
8. Bending, kneeling, or stooping	○ 1	○ 2	○ 3
9. Walking **more than a mile**	○ 1	○ 2	○ 3
10. Walking **several blocks**	○ 1	○ 2	○ 3
11. Walking **one block**	○ 1	○ 2	○ 3
12. Bathing or dressing yourself	○ 1	○ 2	○ 3

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

	Yes	No
13. Cut down the **amount of time** you spent on work or other activities	1	2
14. **Accomplished less** than you would like	1	2
15. Were limited in the **kind** of work or other activities	1	2
16. Had **difficulty** performing the work or other activities (for example, it took extra effort)	1	2

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

	Yes	No
17. Cut down the **amount of time** you spent on work or other activities	1	2
18. **Accomplished less** than you would like	1	2
19. Didn't do work or other activities as **carefully** as usual	1	2

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- 1 - Not at all
- 2 - Slightly
- 3 - Moderately
- 4 - Quite a bit
- 5 - Extremely

21. How much **bodily** pain have you had during the **past 4 weeks**?

- 1 - None
- 2 - Very mild
- 3 - Mild
- 4 - Moderate
- 5 - Severe
- 6 - Very severe

22. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

- 1 - Not at all
- 2 - A little bit
- 3 - Moderately
- 4 - Quite a bit
- 5 - Extremely

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks**...

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
23. Did you feel full of pep?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
24. Have you been a very nervous person?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
25. Have you felt so down in the dumps that nothing could cheer you up?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
26. Have you felt calm and peaceful?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
27. Did you have a lot of energy?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
28. Have you felt downhearted and blue?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
29. Did you feel worn out?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
30. Have you been a happy person?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
31. Did you feel tired?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6

32. During the **past 4 weeks**, how much of the time has **your physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

- ○ 1 - All of the time
- ○ 2 - Most of the time
- ○ 3 - Some of the time
- ○ 4 - A little of the time
- ○ 5 - None of the time

How TRUE or FALSE is **each** of the following statements for you.

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
33. I seem to get sick a little easier than other people	○ 1	○ 2	○ 3	○ 4	○ 5
34. I am as healthy as anybody I know	○ 1	○ 2	○ 3	○ 4	○ 5
35. I expect my health to get worse	○ 1	○ 2	○ 3	○ 4	○ 5
36. My health is excellent	○ 1	○ 2	○ 3	○ 4	○ 5

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ASTHMA QUALITY OF LIFE QUESTIONNAIRE WITH STANDARDISED ACTIVITIES (AQLQ(S))

SELF-ADMINISTERED
ENGLISH VERSION FOR THE UNITED KINGDOM

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MAY 2017

ASTHMA QUALITY OF LIFE QUESTIONNAIRE (S) PATIENT ID: _____
(ENGLISH VERSION FOR THE UNITED KINGDOM)
SELF-ADMINISTERED DATE: _____

Please complete **all** the questions by circling the number that best describes how you have been during the **last 2 weeks as a result of your asthma**.

HOW **LIMITED** HAVE YOU BEEN **DURING THE LAST 2 WEEKS** IN THESE ACTIVITIES **AS A RESULT OF YOUR ASTHMA**?

	Totally Limited	Extremely Limited	Very Limited	Moderate Limitation	Some Limitation	A Little Limitation	Not at all Limited
1. STRENUOUS ACTIVITIES (such as hurrying, exercising, running up stairs, sports)	1	2	3	4	5	6	7
2. MODERATE ACTIVITIES (such as walking, housework, gardening, shopping, climbing stairs)	1	2	3	4	5	6	7
3. SOCIAL ACTIVITIES (such as talking, playing with pets/children, visiting friends/relatives)	1	2	3	4	5	6	7
4. WORK-RELATED ACTIVITIES (tasks you have to do at work*)	1	2	3	4	5	6	7
*If you are not employed or self-employed, these should be tasks you have to do most days.							
5. SLEEPING	1	2	3	4	5	6	7

HOW MUCH **DISCOMFORT OR DISTRESS** HAVE YOU FELT **DURING THE LAST 2 WEEKS**?

	A Very Great Deal	A Great Deal	A Good Deal	Moderate Amount	Some	Very Little	None
6. How much discomfort or distress have you felt over the last 2 weeks as a result of CHEST TIGHTNESS?	1	2	3	4	5	6	7

IN GENERAL, **HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS** DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
7. Feel CONCERNED ABOUT HAVING ASTHMA?	1	2	3	4	5	6	7
8. Feel SHORT OF BREATH as a result of your asthma?	1	2	3	4	5	6	7
9. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO CIGARETTE SMOKE?	1	2	3	4	5	6	7
10. Experience a WHEEZE in your chest?	1	2	3	4	5	6	7
11. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF CIGARETTE SMOKE?	1	2	3	4	5	6	7

HOW MUCH **DISCOMFORT OR DISTRESS** HAVE YOU FELT **DURING THE LAST 2 WEEKS**?

	A Very Great Deal	A Great Deal	A Good Deal	Moderate Amount	Some	Very Little	None
12. How much discomfort or distress have you felt over the last 2 weeks as a result of COUGHING?	1	2	3	4	5	6	7

IN GENERAL, **HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS** DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
13. Feel FRUSTRATED as a result of your asthma?	1	2	3	4	5	6	7
14. Experience a feeling of CHEST HEAVINESS?	1	2	3	4	5	6	7

IN GENERAL, **HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS** DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
15. Feel CONCERNED ABOUT THE NEED TO USE MEDICATION for your asthma?	1	2	3	4	5	6	7
16. Feel the need to CLEAR YOUR THROAT?	1	2	3	4	5	6	7
17. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO DUST?	1	2	3	4	5	6	7
18. Experience DIFFICULTY BREATHING OUT as a result of your asthma?	1	2	3	4	5	6	7
19. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF DUST?	1	2	3	4	5	6	7
20. WAKE UP IN THE MORNING WITH ASTHMA SYMPTOMS?	1	2	3	4	5	6	7
21. Feel AFRAID OF NOT HAVING YOUR ASTHMA MEDICATION AVAILABLE?	1	2	3	4	5	6	7
22. Feel bothered by HEAVY BREATHING?	1	2	3	4	5	6	7
23. Experience asthma symptoms as a RESULT OF THE WEATHER OR AIR POLLUTION OUTSIDE?	1	2	3	4	5	6	7
24. Were you WOKEN AT NIGHT by your asthma?	1	2	3	4	5	6	7
25. AVOID OR LIMIT GOING OUTSIDE BECAUSE OF THE WEATHER OR AIR POLLUTION?	1	2	3	4	5	6	7

IN GENERAL, **HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS** DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
26. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO STRONG SMELLS OR PERFUME?	1	2	3	4	5	6	7
27. Feel AFRAID OF GETTING OUT OF BREATH?	1	2	3	4	5	6	7
28. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF STRONG SMELLS OR PERFUME?	1	2	3	4	5	6	7
29. Has your asthma INTERFERED WITH GETTING A GOOD NIGHT'S SLEEP?	1	2	3	4	5	6	7
30. Have a feeling of FIGHTING FOR AIR?	1	2	3	4	5	6	7

HOW LIMITED HAVE YOU BEEN **DURING THE LAST 2 WEEKS**?

	Severely Limited Most Not Done	Very Limited	Moderately Limited Several Not Done	Slightly Limited	Very Slightly Limited Very Few Not Done	Hardly Limited At All	Not Limited Have Done All Activities
31. Think of the OVERALL RANGE OF ACTIVITIES that you would have liked to have done during the last 2 weeks. How much has your range of activities been limited by your asthma?	1	2	3	4	5	6	7

HOW LIMITED HAVE YOU BEEN **DURING THE LAST 2 WEEKS**?

	Totally Limited	Extremely Limited	Very Limited	Moderate Limitation	Some Limitation	A Little Limitation	Not at all Limited
32. Overall, among ALL THE ACTIVITIES that you have done during the last 2 weeks, how limited have you been by your asthma?	1	2	3	4	5	6	7

DOMAIN CODE:

Symptoms: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 29, 30
Activity Limitation: 1, 2, 3, 4, 5, 11, 19, 25, 28, 31, 32
Emotional Function: 7, 13, 15, 21, 27
Environmental Stimuli: 9, 17, 23, 26

Appendix J. Woman's questionnaire

For clinical visit participants, version 09.04.2021

Centre ☐☐☐

ID ☐☐☐☐☐

1. What is today's date? Day ☐☐ Month ☐☐ Year ☐☐

2. Do you have regular periods? Tick one box only

- Yes ☐ 1
- No, they have never been regular ☐ 2
- No, they have been irregular for a few months ☐ 3
- No, my periods have stopped ☐ 4

3. What is the usual interval between your periods *or* what *was* the usual interval between your periods before they became irregular or stopped?
(from the first day of one period to the first day of the next) Tick one box only

- Less than 24 days ☐ 1
- 24-26 days ☐ 2
- 27-29 days ☐ 3
- 30-32 days ☐ 4
- 33-35 days ☐ 5
- more than 35 days ☐ 6

4. When was your last period? Day ☐☐ Month ☐☐ Year ☐☐

(Please fill in the date of the first day of your last period)

4.1 If you do not remember your last menstrual period, and your periods have stopped, how old were you when you had your last menstruation? Age in Years ☐☐

5. How many periods have you had in the last 12 months? Number ☐☐

5.1 If you had periods in the last 12 months

5.1.1 Is your menstrual cycle often (more than twice a year) more than 35 days? No ☐ Yes ☐

5.1.2 Have your periods been irregular over the last 12 months? No ☐ Yes ☐

If your periods have been irregular over the last 12 months

5.1.2.1 For how long have your periods been irregular? Months ☐☐

5.2 If you had <u>no period</u> in the last 12 months

5.2.1 What statement best describes the reason you have not had a period in the last 12 months? Tick one box only

- Menopause ☐ 1
- Hysterectomy (womb removed) ☐ 2
- Ovaries removed ☐ 3
- Because I have been taking treatments (eg hormonal IUD, contraceptive implants, chemotherapy) ☐ 4
- Other please describe_____ ☐ 5

5.2.2. Did your periods become irregular before they stopped? No ☐ Yes ☐

If YES

5.2.2.1 How old were you when they became irregular? Years ☐☐

<u>FOR ALL WOMEN (CONTINUED)</u>

6. Have you ever had a hysterectomy (your womb removed)? No ☐ Yes ☐

If NO go to question 7,
If YES:–

6.1 How old were you when you had a hysterectomy? Years ☐☐

6.2 What was the **<u>main</u>** reason you had a hysterectomy? Tick one box only

- Heavy or painful or irregular periods ☐ 1
- Fibroids, (with or without heavy, painful or irregular periods) ☐ 2

Cancer of the womb (endometrium)	☐ 3
Cancer of the ovary	☐ 4
Cancer of the cervix	☐ 5
Vaginal prolapse	☐ 6
Don't know/don't wish to say	☐ 7
Other……	☐ 8

7. Have you ever had one or both ovaries removed? Tick one box only

Never	☐ 1
Yes, one ovary	☐ 2
Yes, two ovaries	☐ 3
Don't know	☐ 4

If you have had one or both ovaries removed –

7.1 How old were you when you had your ovary/ies removed?
(Fill-in 2 lines if you had your 2 ovaries removed at a different age)

Years

8. Has a doctor or health professional <u>ever told you</u> have….

	No	Yes		
8.1 Ovarian cyst or cysts	☐	☐	**If Yes :** ↳ **8.1.1** How old were you when a doctor told you you had ovarian cyst/s?	Years ☐☐
8.2 Polycystic ovaries or polycystic ovarian syndrome (PCOS)	☐	☐	**If Yes :** ↳ **8.2.1** How old were you when a doctor told you you had polycystic ovaries or PCOS?	Years ☐☐
8.3 Fibroids	☐	☐	**If Yes :** ↳ **8.3.1** How old were you when a doctor told you you had fibroids?	Years ☐☐
8.4 Endometriosis	☐	☐	**If Yes :** ↳ **8.4.1** How old were you when a doctor told you you had endometriois?	Years ☐☐
8.5 Osteoporosis	☐	☐	**If Yes :**	Years ____

↳ **8.5.1** How old were you when a doctor told you you had osteoporosis? ☐☐

9. Some women experience hot flushes, flashes and/or night sweats around the time of the menopause, even when they are having menstrual cycles. Have you ever had either of these symptoms in the last 12 months? No ☐ Yes ☐

If NO go to question 10,
If YES:

9.1 How old were you when these symptoms started? Years ☐☐

10. Have you ever taken hormonal contraceptives (eg the pill, patches, injections, implants, coil impregnated with hormone eg. Mirena)? No ☐ Yes ☐

11. **Have you ever taken hormonal treatment for the menopause (tablets, cream, patches, vaginal creams or vaginal pessaries)?** No ☐ Yes ☐

12. **Are you <u>currently</u> taking any of the following treatments**

		No	Yes	If yes, number of months using this treatment
12.1	**Hormonal treatment for contraception (eg 'the pill', hormonal coil, ...)**	☐	☐	☐☐☐
12.2	**treatment of menopausal symptoms (eg HRT)**	☐	☐	☐☐☐
12.3	**DHEA (dehydroepiandosterone)**	☐	☐	☐☐☐
12.4	**to treat gynaecological disorders, or other treatment**	☐	☐	☐☐☐

If you are currently taking any of these treatments:

12.5 Name of treatment you are currently taking _____

FOR ALL WOMEN (CONTINUED)

13. If you have <u>ever</u> used any hormonal treatment for contraception or the menopause

For each period of age from age 10 years to now, please tick the years when you take the following types of hormonal treatments.

If you have taken then off and again, please tick the periods when you used them, leave blanks for years when you did not use them, and tick again the years when you used them again.

If you used several treatments at the same age, tick them all in the same age column

If you have <u>never</u> used the treatment, tick the box in first column

The last two lines are shown as an example

	If you have **never used** this treatment, thik 1st box	***If you have ever used this treatment, tick the ages when you have been using it***																				
age		10-12	13-15	16-18	19-21	22-24	25-27	28-30	31-33	34-36	37-39	40-42	43-45	46-48	49-51	52-54	55-57	58-60	61-63	64-66	67-69	70-72	73-75
Hormonal contraceptives																							
Tablets																							
Patches																							
Vaginal ring																							
Injections / implants																							
Coil impregnated with hormones																							
Hormonal treatment for the menopause																							
Oral preparations																							
Patches																							
Vaginal preparations																							
Exemple, traitment X	**X**																						
Exemple, traitment Y																—		—	—	—	—	—	

FOR ALL WOMEN (CONTINUED)

14. How often do you usually use the following personal products?

Tick one box per product

		Never	<1 day/ week	1-3 days/ week	4-7 days/ week	>1 time/ day
13.1	Perfume spray	☐	☐	☐	☐	☐
13.2	Perfume (not spray)	☐	☐	☐	☐	☐
13.3	Deodorant spray	☐	☐	☐	☐	☐
13.4	Deodorant stick	☐	☐	☐	☐	☐
13.5	Hair spray	☐	☐	☐	☐	☐
13.6	Moisturising cream	☐	☐	☐	☐	☐
13.7	Lotions	☐	☐	☐	☐	☐
13.8	Cleansing cream	☐	☐	☐	☐	☐
13.9	Nail polishes	☐	☐	☐	☐	☐
13.10	Nail polishes remover	☐	☐	☐	☐	☐
	code for fieldworker	1	2	3	4	5

		Never	less than once a month	more than once a month
13.11	Hair dye	☐	☐	☐
13.12	Hair bleach	☐	☐	☐
	code for fieldworker	1	2	3

Appendix K. Male's questionnaire (Aging Males Symptom Scale, AMS)

AMS Questionnaire

Which of the following symptoms apply to you at this time? Please, mark the appropriate box for each symptom. For symptoms that do not apply, please mark "none".

	Symptoms:	none	mild	moderate	severe	extremely severe
	Score =	1	2	3	4	5
1.	**Decline in your feeling of general well-being** (general state of health, subjective feeling)	☐	☐	☐	☐	☐
2.	**Joint pain and muscular ache** (lower back pain, joint pain, pain in a limb, general back ache)	☐	☐	☐	☐	☐
3.	**Excessive sweating** (unexpected/sudden episodes of sweating, hot flushes independent of strain)	☐	☐	☐	☐	☐
4.	**Sleep problems** (difficulty in falling asleep, difficulty in sleeping through, waking up early and feeling tired, poor sleep, sleeplessness)	☐	☐	☐	☐	☐
5.	**Increased need for sleep, often feeling tired**	☐	☐	☐	☐	☐
6.	**Irritability** (feeling aggressive, easily upset about little things, moody)	☐	☐	☐	☐	☐
7.	**Nervousness** (inner tension, restlessness, feeling fidgety)	☐	☐	☐	☐	☐
8.	**Anxiety** (feeling panicky)	☐	☐	☐	☐	☐
9.	**Physical exhaustion / lacking vitality** (general decrease in performance, reduced activity, lacking interest in leisure activities, feeling of getting less done, of achieving less, of having to force oneself to undertake activities)	☐	☐	☐	☐	☐
10.	**Decrease in muscular strength** (feeling of weakness)	☐	☐	☐	☐	☐
11.	**Depressive mood** (feeling down, sad, on the verge of tears, lack of drive, mood swings, feeling nothing is of any use)	☐	☐	☐	☐	☐
12.	**Feeling that you have passed your peak**	☐	☐	☐	☐	☐
13.	**Feeling burnt out, having hit rock-bottom**	☐	☐	☐	☐	☐
14.	**Decrease in beard growth**	☐	☐	☐	☐	☐
15.	**Decrease in ability/frequency to perform sexually**	☐	☐	☐	☐	☐
16.	**Decrease in the number of morning erections**	☐	☐	☐	☐	☐
17.	**Decrease in sexual desire/libido** (lacking pleasure in sex, lacking desire for sexual intercourse)	☐	☐	☐	☐	☐

Have you got any other major symptoms? Yes ☐ No ☐

If Yes, please describe: _____

_____

THANK YOU VERY MUCH FOR YOUR COOPERATION

Bibliographic reference(s) of the original questionnaire

Heinemann LAJ. Aging Males Symptoms Scale (AMS). Development of the scale. June 2006

Heinemann LAJ, Zimmermann T, Vermeulen A, Thiel C, Hummel W. A new, aging males' symptoms' rating scale. The Aging Male 1999;2:105-114

Heinemann LAJ, Thiel Ch, Assmann A, Zimmermann T, Hummel W, Vermeulen A. Sex differences of „climacteric symptoms" with increasing age? A pooled analysis of cross-sectional population-based surveys. The Aging Male 2000; 3:124-131

Appendix L. Measurement of Fractional Exhaled Nitric Oxide (FeNO)

Aim

The aim of the procedure is to measure exhaled nitix oxide (Fraxtional exhaled Nitric Oxide, FeNO) in the breath of the participants. FeNO measurement is a quantitative and non-invasive measure of airway inflammation.

Responsibility

NIOX VERO® may only be operated as directed in this procedure and in the User Manual. The center-PI is responsible for ensuring that all employees have received correct training in this procedure before performing the measurement. All fieldworkers operating the instrument must watch the educational videos below as part of the training.
https://www.niox.com/en/videos/sophie-toor-demo
https://www.niox.com/en/videos/educational-videos
http://www.aerocrine.com/en/niox-mino/Videowindow.html

About the instrument

NIOX VERO®measures Nitric Oxide in human breath (Fractional exhaled Nitric Oxide, FeNO) and Nasal Nitric Oxide (nNO) in the aspirated air from the nasal cavity.

FeNO is increased in some airway inflammatory processes such as asthma and decreases in response to anti-inflammatory treatment. FeNO measurements with NIOX VERO are quantitative, non-invasive, simple and safe and should be used as part of regular assessment and monitoring of patients with these conditions.

Deviations and accuracy

NIOX VERO® should be kept away from:

- Mobile phones, computers and other electromagnetic sources
- Open windows, direct sun and radiators
- Use of substances containing alcohol close to the NIOX VERO instrument may cause erroneous measurements results.

Procedure

Exhaled nitric oxide levels should be measured before other spirometric assessment and before skin prick testing. For ECRHSIV and EPHOR, exhaled nitric oxide measures should be made after completion of the 'Getting ready for FENO, spirometry, reversibility and bioimpedence' questionnaire.

Equipment

- NIOX VERO®
- Single-use filter

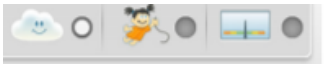
Preparing the participant for measurement

For one hour prior to measurement participants should refrain from

- Smoking for one hour
- Eating or drinking for one hour
- Strenuous exercise

Demo for instructing the participant

Select the **Animation** button on the main manu. 

Select which animation to use (Cloud, Ballon or Meter). 

Select the **Demo** button. 

Select **Forward** button  to move to the following sequence

Select **Undo** button  to close the demonstration and return to animation select.

Performing the NO-measurement

1. Touch the screen on NIOX VERO®to get out of standby or sleep mode.
2. Make sure that the exhalation time is in the **preferred mode of 10 seconds** (see below).
3. Attach the patient filter to the breathing handle. Make sure to twist the patient filter in place until it clicks into place.
4. Measurements are made in the sitting position. Place the screen in front of the participants so that they can see the display. This will help them to know if they are exhaling at the correct speed.
5. Participants are asked to:

- Empty their lungs through a single long exhalation
- Close the lips around the mouthpiece on the patient filter so that no air leakage occurs, and take a deep breath until they reach total lung capacity. During inhalation, the cloud on the display moves upwards.
- Without delay participants should then exhale through the mouthpiece, slowly and steadily in such a way as to comply with the audio and visual feedback (keep the ‘cloud’ between the two horizontal lines) from the device.
    - A continuous sound indicates correct pressure with a frequency proportional to the pressure.
    - An intermittent high frequency sound – too strong pressure.
    - An intermittent low frequency sound – too weak pressure.
- Exhale until the cloud has passed the flag. The instrument will analyse the sample and generate a result in approximately one minute.

Performing NO-measuremet in the 6 seconds mode

1. Select **Settings** in the main menu. 

2. Select **Modes configuration**. 
3. Check the **10s/6s** icon to enable using the 6s mode. Uncheck to disable. Press **OK**. 

FeNO is measured at the plateau of expiration and given in parts per billion (ppb). This measurement will be given on the screen and should be recorded.

If the participant is unable to complete the test at the first attempt this should be repeated. No more than nine attempts should be made. The number of attempts should be recorded.

When the test is complete the mouthpiece should be removed. A new one should be inserted prior to the next test.

Cleaning of instrument

Clean the breathing handle and the instrument with a cloth dampened with water or a mild soap solution.

Do not use disinfectants or wipes containing alcohol, or spray detergents. These might permanently damage the sensor and instrument.

Patient filters are labeled for single use only. Always use a new patient filter for each patient. Reuse between patients could increase the risk of cross-contamination or cross-infection.

Maintenance of instrument

NIOX VERO® Breathing handle

The breathing handle contains a NO scrubber which can be used for 1000 measurements or one year, whichever comes first. The breathing handle view (figure 1) is used for viewing the status of the breathing handle and for resetting breathing handle usage parameters.

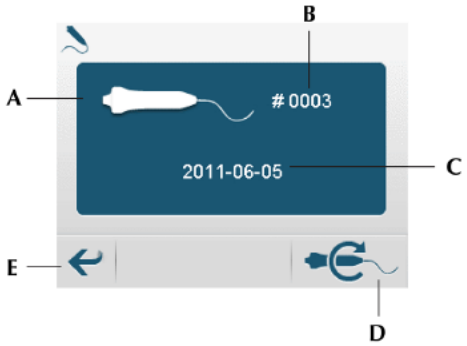


Figure 1 Breathing handle view, where A = Breathing handle symbol, B = Remaining number of measurements, C = Expiration date, D = Breathing handle reset button, and E = Return button.

NIOX VERO® instrument

The operational life-time of the NIOX VERO® instrument is a maximum 5 years from first use or 15 000 measurements, whichever comes first. The user is prompted for expiry parameters via the device display. It is not possible to perform further measurements after expiry, although stored measurement data can still be retrieved.

NIOX VERO® Sensor

Operational life-time of the NIOX VERO® is maximum 12 months after opening package and installation in NIOX VERO® or until expiration date as stated on the sensor, whichever comes first.
The sensor will expire after the pre-programmed number of measurements have been depleted, or after one year (whichever comes first). When there is less than 10% of the number of the measurements left, or less than two weeks of use remaining, a message is shown on the display.

After mounting a new sensor it is recommended to wait for 3 hours before use.

This device is not user serviceable. Do not open the device except for sensor or battery replacement as outlined in this manual. Never attempt to perform sensor or battery replacement while the device is in operation. Do not modify the handle tube. For troubleshooting, see Usermanual.

References

User Manual – NIOX VERO® Airway Inflammation Monitor. CIRCASSIA. EPM000167.book (niox.com) (accessed 22.06.2021).

Log of changes in settings of the instrument

Date	Version	Initials	Changes performed

Appendix M. Collection, pre-processing and storage of blood samples

1. Long-term study: Collection, pre-processing and storage of blood (source: ECRHSIV)

Introduction

In the EPHOR long-term study, blood samples collected in ECRHSIV will be used for analysis of leucocytes (white blood cells, WBC), differential count of leukocytes, and the inflammation markers CC-16 and YKL-40 (markers of susceptibility). Blood will be collected using standard venesection techniques (Annex 1 Best practices in phlebotomy). Staff should be trained and insured to carry out venepuncture according to local requirements.

Tube	**Colour**	**Size**	**Number of inversions to mix**	**Purpose**	**Overview of handling**	**Storage at -20oC**
Gel Serum Separator BD SST™	Gold	7mL	5	Inflammation markers	Allow to clot for 60 minutes. Centrifuge for 15 minutes at 3000 rpm and prepare aliquots	Two aliquotsof 2mL Starstedt tube with screw cap Remaining aliquots of 1mL Into storage boxes
EDTA	Lavender	6mL	10	WBC and differential count	-	Store directly

Equipment required

Clinical gloves
Sharps bin
Tourniquet
Cotton
Wool swabs
Plastic storage tubes 6 X 2ml
Small receiver
Spot plasters/micropore
Blood spillage kit
Barcode stickers
Checklist for order of draw
Washable pillow
Suitable couch or chair (with arms and without wheels)

Tube rack (if the field)
BD Vacutainer™ Plastic Blood Collection Tubes (see table above)

All study project centers are asked to use the same BD Vacutainer Plastic Blood Collection Tubes where possible. These contain either anticoagulant or clot activator and therefore require immediate mixing following collection.

Explain the procedure to the participant and ascertain if they may feel faint when giving a blood sample. If so, ask them to lie down. Otherwise they should be positioned comfortably with their arm straight and resting on a hard surface or pillow.

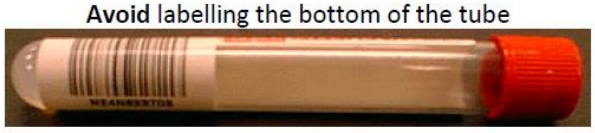
Procedure

- Wash your hands and apply gloves (Annex 2 Instructions for hand washing)
- Using a tourniquet, locate a suitable vein for venepuncture (median cubital, basilic or cephalic) Insert vacutainer needle into holder.
- Insert needle into vein, insert first bottle into vacutainer holder, pushing it firmly into place and ensuring it pierces rubber stopper allowing the vacuum to be completely filled.
- Remove bottle from holder, keeping needle situated in the vein and continue to fill the blood bottles in correct order of draw. Mix each blood tube as required before inserting a new tube. The exchange of vacutainers should be smooth and the final blood tube removed prior to the needle being withdrawn from the vein.
- When draw is complete, remove the tourniquet and gently withdraw the needle from the vein and place cotton wool swab firmly over the puncture site. Apply pressure to the puncture site for approximately half-a-minute.
- Dispose of sharps directly into a sharps bin and transfer other contaminants to a clinical waste bag. Ensure that the outside of the blood bottles are free from blood. Label the EDTA tube with one of the subject's ID bar-coded stickers. Ensure that the sticker is aligned lengthways and at the top of the blood tube, that is, with the longer end of the sticker placed lengthways along the tube so that the entire barcode and ID number are visible, flat and not obscured by any overlap.

Please note that these pictures are not based on the barcode labels that we expect to be able to provide

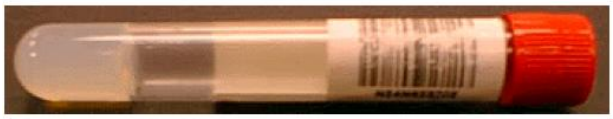
Incorrect methods

Avoid labelling the bottom of the tube



Do **not** wrap labels around the tube

Avoid wrinkles, folds or tears in label



Avoid incomplete or illegible labels

It is not necessary to barcode label the serum collection tubes as they will be disposed of after centrifugation (carefully write the ID code onto the serum bottles).

Preparation of serum sample

Equipment

Fridge -20ºC and -80°C freezers (with thermometer)
Swing head or fixed angle centrifuge
2ml (Sarstedt) storage tubes – (or tubes suitable for -20ºC and -80°C freezing and that can fit 24x13mm labels) and lids
Sarstedt tube storage boxes
Laboratory safety equipment (lab coat, glasses, gloves)
Disposable graduated 3ml pipettes
Barcode stickers
Barcode reader
Laboratory sample logbook
Results sheet

Procedure

Stand the Gel separator tubes upright in a rack and let them clot for at least 60 minutes standing upright in a rack.

Spin the tube for 3000 rpm for 15 minutes. Samples may be stored in a fridge overnight before they are centrifuged. This should only be the case if for example it is late in the evening and the technician needs to go home. Samples should be spun first thing the following morning.

Pipette the serum and transfer it into storage microtubes with rubber seal cap (2 ml each) SARSTEDT

Prepare these aliquots in the following order:

- 2 x 2mL
- and all remaining aliquots as 1mL

Sample storage tubes must be labeled with the correct ID barcode label. Stick the label lengthways on the tube. **Do not wrap the label around the tube** (ensure that the whole of the bar code and ID are visible).

Store the sample tubes in a carefully labeled storage box at -20ºC making appropriate record in the sample log book.

The serum samples for analysis of the inflammation markers CC-16 and YKL-40 is stored at -80ºC until shipment to ISGlobal for analysis.

It is important to maintain an **impeccable sample logbook**. Copies of it will be required during sample shipment. An example of a logbook page is given on the next page.

Sample Log Book

Study: European Community Respiratory Health Survey / EPHOR
Centrifugation Speed: 3000 rpm
Centrifugation Time: 15 minutes
Freezer Temperature: -20 °C (-80°C for one serum sample)
Samples: Serum, whole blood (not to be spun)

Barcode	ID	Date taken	Date spun	Number 2mL aliquots	Number 1mL aliquots	Whole blood N/Y	Storage Box Number	Location
B20053S	*12453*	*25/12/12*	*25/12/12*	*2*			*2*	*B5-B6*
B20053S	*12453*	*25/12/12*	*25/12/12*		*5*		*3*	*C2-C6*
B20053S	*12453*	*25/12/12*	*25/12/12*			*Y*	*5*	
B20054S	*12942*	*01/01/13*	*01/01/13*	*1*			*2*	*B7*
B20054S	*12942*	*01/01/13*	*01/01/13*		*3*		*3*	*C7-C9*
B20054S	*12942*	*01/01/13*	*01/01/13*			*N*	*-*	

Freezer temp check	Date	Initials

This page can be photocopied and a bound file of log pages prepared for use in the project. The data can also be stored electronically (in the same format).

At least once a week a record of the freezer temperature should be noted in the logbook.

Further instructions on transport of samples to the laboratory will be provided at a later date.

2. Short-term study: Collection, pre-processing and storage of **blood** (source: EPHOR WP3)

Introduction

In the short-term study, blood samples will be used for analysis of metals, cotinine (marker of nicotin use), white blood cell count (WBC) and differential count, cytokines, oxidative damage (8-hydroxydeoxyguanosine (8-OHdG), mitochondrial DNA copy number, telomere length, genome-wide DNA methylation, sequence specific DNA methylation (pyrosequencing), targeted RNA expression, cell-free DNA (cfDNA) methylation, proteomics and

Note: The exact time of sampling will be discussed with WP6 and 7 leaders.

Materials needed

Collection:	-10 mL Vacutainer® EDTA tubes -2.5 mL PAXgene® blood RNA tube -6 mL Vacutainer® blood collection tube for trace element testing - 4 mL Vacutainer® serum tube -rack for tubes -BD Vacutainer® Safety-Lok blood collection set – or …. -Powder-free disposable gloves -70% alcohol swabs for skin disinfection -Garrottes/tourniquets -Adhesive bandages or tapes -Container for disposal of used needles after venipuncture -Labels: country ID – participant ID – sample ID (including date and time of collection) -Barcode scanner -Datasheet for information on time of sample collection and time since last meal
Pre-processing:	-Bench top Centrifuge (Eppendorf/Sigma) -Pipettes and tips -Nalgene™ General Long-Term Storage Cryogenic Tubes (Catalogue number: 5000-0020; Volume- 2 mL; ThermoFisher) -Labels: country ID – participant ID – sample ID (including date and time of collection)
Storage:	-Storage box -Freezer -80 °C

Collection of blood

Note: Clean space preferable; some experiments need sterile condition.

Participants are asked to refrain from drinking, eating and smoking before collection.

Type of tube	Number of tubes	Volume of tubes (mL)
Vacutainer® EDTA	4	10
PAXgene® blood RNA tube	1	2.5
Vacutainer® blood collection tube for trace element testing	1	6
Vacutainer® serum tube	1	4
Total volume		52.5

Note: Additional clinical parameters might be studied, thus an additional heparin tube might be collected. This will be added to the protocol after discussion with the clinical lab.

Instruction for collection

- Label tubes
- Select tube for sample collection (first BD Vacutainer® EDTA, then BD Vacutainer® blood collection tube for trace element testing, end with PAXgene tube). Place sample tubes on a rack in order of collection.
- Blood samples are collected according to WHO's best practices in phlebotomy (annex 1).
- Assemble a blood collection set with 12-inch tubing into a BD Vacutainer® One Use Holder. Be sure that blood collection set is firmly attached to holder and does not unthread during use.
- Hands are washed (see annex 2) and a mask is worn. Patients are identified and prepared.
- Select site for venipuncture; Apply tourniquet.
- Prepare a venipuncture site with appropriate antiseptic. Do not palpate the venipuncture site after cleansing.
- Perform venipuncture with limb downward and tube stopper up (for prevention of backflow).
- Push tube onto non-patient-end (NP-end) of needle in one swift action. Hold tube on NP-end during drawing.
- Remove tourniquet as soon as blood appears in the last tube.
- Do not allow the contents of the tube to contact the stopper or end of the needle during the procedure.
- Allow vacuum to be exhausted prior to removing the tube from the NP (non-patient) end of the needle.
- Give the participant an adhesive bandage or tape to apply to the puncture site.
- Discard the used equipment into a puncture-resistant container, discard sharps and broken glass into the sharps container and discard items containing blood or body fluids into the infectious waste.
- Remove gloves and wash hands.

- Mix specimen tubes with additives, by slowly inverting the tube 8 to 10 times immediately after blood collection.
- Fill-in datasheet with information on time of collection and covariates (see annex 3 Datasheet).

Pre-processing and storage of blood at site of collection

Note: sterile condition required for most of the following steps.

Time allowed from collection and processing: 2-6 h.

Note: It is very important to minimize the time between collection and separation of plasma; We want to study cell free DNA/protein and prolonged storage of blood after collection without processing will result in haemolysis and cell death; Thereby, resulting in release of cellular DNA and proteins into the matrix and altering the profile.

- 1 PAXgene RNA tube of 2.5 mL is kept for 1 h at ambient temperature and then stored at -80 °C for ddPCR analysis (STAMI).
- 1 Vacutainer® blood collection tube for trace element testing of 6 mL to be stored at 20°C (KI).
- Blood from EDTA tubes is aliquoted in 6 cryo-vials of 2 mL (1 mL per vial). 2 vials are sent for differential count of cells at the site of collection (no storage), 4 vials are immediately stored at -80 °C (KUL and KI).
- For preparation of blood collected in Gel Serum Separator tubes, see below.
- The remaining blood samples from EDTA tubes are centrifuged (2000 x g, 10 minutes, at 20 °C). (**Note**: Shorter time between collection and centrifugation should be ideal)
- After centrifugation, plasma is aliquoted in 8 cryo-vials of 2 mL (1 mL per vial) (ISGlobal and KUL). All vials are carefully labeled.
- Store all cryo-vials at -80 °C.
- Leave the cellular fraction (pellet) in the EDTA tube and store at -80 °C (KUL).

Preparation of serum sample

Equipment

Fridge -20°C and -80°C freezers (with thermometer)
Swing head or fixed angle centrifuge
2ml (Sarstedt) storage tubes – (or tubes suitable for -20ºC and -80°C freezing and that can fit 24x13mm labels) and lids
Sarstedt tube storage boxes
Laboratory safety equipment (lab coat, glasses, gloves)
Disposable graduated 3ml pipettes
Barcode stickers
Barcode reader
Laboratory sample logbook
Results sheet

Procedure

Stand the Gel separator tubes upright in a rack and let them clot for at least 60 minutes standing upright in a rack.

Spin the tube for 3000 rpm for 15 minutes. Samples may be stored in a fridge overnight before they are centrifuged. This should only be the case if for example it is late in the evening and the technician needs to go home. Samples should be spun first thing the following morning.

Pipette the serum and transfer it into storage microtubes with rubber seal cap (2 ml each) SARSTEDT . Prepare these aliquots in the following order:

- 2 x 2mL
- and all remaining aliquots as 1mL

Store the sample tubes in a carefully labeled storage box at -20ºC. The serum samples for analysis of the inflammation markers CC-16 and YKL-40 is stored at -80ºC until shipment to ISGlobal for analysis.

Note from WP3: Please note that the final method for cotinine will be adapted based on method development, optimization and validation. Before the start of the experiments, the developed and validated protocol will be made available. The protocol for collection, pre-processing and storage of blood for cotinine analysis described here is therefore only a limited outline.

3. Measurement of leukocytes (WBC count) and differential counts using the "Point-of-care" HemoCue® WBC DIFF System

The HemoCue WBC DIFF System is a Point-OF-Care instrument that are used to monitor white blood cell (WBC) count and a 5-part differentiation (number of lymphocytes, eosinophils, neutrophils, basophils and monocytes) in less than 5 minutes [1].

The instrument and its' requirements

The HemoCue WBC Diff system is a Point-Of-Care device weighing 600g with approximate dimensions of 13cm x17cmx12cm (Figure 1). It can be powered by 6 AA drycell batteries or AC electricity mains. It requires 10 µl of blood in a dye-filled cuvette accessory and produces a differential in less than 5 minutes.

The HemoCue® WBC DIFF System has been reported to be reliable for venous samples for both total white cell count (WBC) [2,3] and the differential count (lymphocytes, eosinophils, neutrophils, basophils and monocytes [3]. It was also reported to provide reliable and accurate counts of eosinophils in healthy controls and subjects with asthma and COPD [4] and WBC, neutrophils and lymphocytes in samples from patients with leukemia [5].

For start-up, set-up, and how to prepare a patient test, see Operating Manual [1].

Figure 1 The HemoCue® WBC DIFF System with microcuvettes.

How does it work?

A microcuvette serves as a pipette, sample container and reaction chamber. A blood sample of approximately 10 µL is drawn into a cavity by capillary action. The blood dissolves the dry content in the microcuvette, the erythrocytes are hemolyzed and the leukocytes are stained with methylene blue.

According to the producer the fixed volume used in the test is defined by the depth of the cavity in the microcuvette and the size of the image (no. of pixels). A camera moved by a high precision motor to achieve an exact and repeatable movement is used to capture images of the stained white blood cells. As the camera moves throughout the cavity of the microcuvette, it takes more than 30 images of each cell. Imaging technology is used to decide when a cell is in focus, and all focused cells are merged into one final image. The cells are classified as neutrophils, lymphocytes, monocytes, eosinophils, basophils, pathological white blood cells (blasts and immature granulocytes) and others. The analyzer will automatically flag all samples containing pathological white blood cells.

Requirements

The users of the device should be adequately trained about test requirements, performance, limitations, and potential interferences (see Operating Manual [1]).

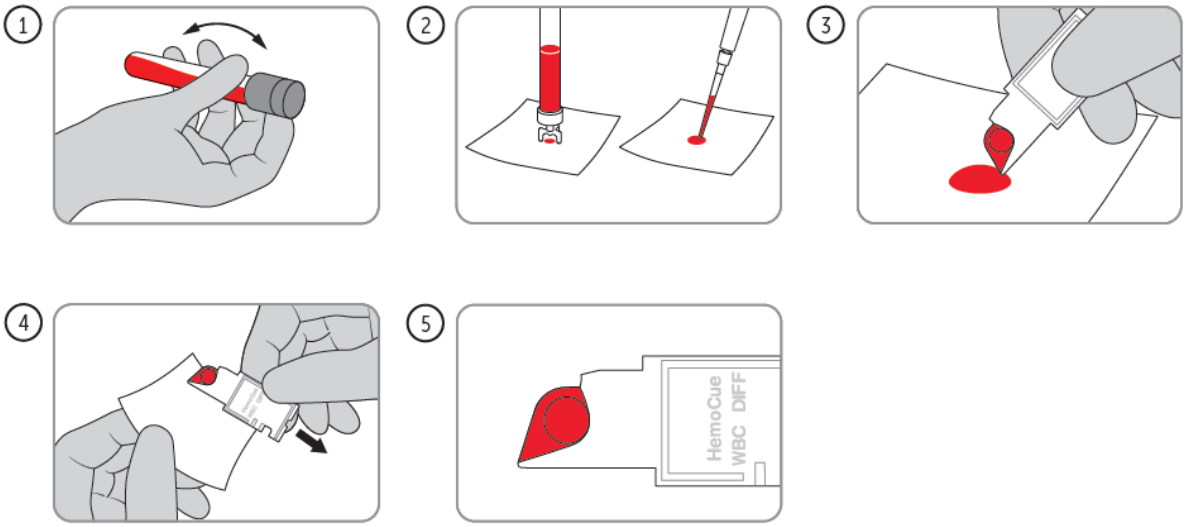
Equipment required
In addition to the equipment needed for venesection in "B", the following is required:

- HemoCue WBC DIFF System
- HemoCue WBC DIFF Microcuvettes
- Pipette or other transfer device
- Lint-free tissue (non-fraying)
- Hydrophopic surface

Procedure

i) Collection of blood sample

1. Venous sample from a Vacutainer® EDTA tupe collected in step B must be mixed thoroughly using a roller mixer for 1-2 minutes or invert the tube 10-20 times by hand. Venous blood samples can be stored at room temperature (18 – 30 C) and analyzed within eight hour after sample collection.
2. Place a drop of blood onto a hydrophobic surface using suitable transfer device.
3. Fill the microcuvette in one continuous process with 10 µL blood.
4. Wipe off excess blood from the outside of the microcuvette with a clean, lint-free wipe. Do not touch the open end of the microcuvette. If air bubbles are present in the filled microcuvette, it must be disccarded.
5. Start the measurement as soon as possible but no later than 1 minute after filling the microcuvette (se below).



ii) Test, storing and printing of the results

1. Make sure that the “Insert cuvette symbol” is shown (1).
2. Push the button for patient test and place the microcuvette in the cuvette holder (2).
3. Start measurement as soon as possible but no later than 1 minute after filling the microcuvette by gently pushing the cuvette holder to its measuring position (3).
4. During measurement the “Measuring window” is shown (4).
5. Read off the results within five minutes (5).
6. The result will be printed automatically (6).
6. Dispose of the microcuvette to a clinical waste bag.

Note: Only current result can be transferred directly to the printer. Stored results cannot be printed.

1 2 3 4

5 6

Cleaning, disinfection and maintenance

The cuvette holder must be cleaned after each day of use.
1. Turn of the analyser. Place the cuvette moving arm in loading position.
2. Remove cuvette holder by lifting it straight up.
3. Clean the cuvette holder with alcohol (20-70%) or mild detergent. Note: Do not autoclave.
4. Wait 15 min before replacing the cuvette holder.
5. Place the cuvette moving arm in loading position before replacing the cuvette holder. Clean the cover with alcohol (20-70%) or mild detergent.

For cleaning of optical parts, see Operating Manual [1].

Calibration and quality control

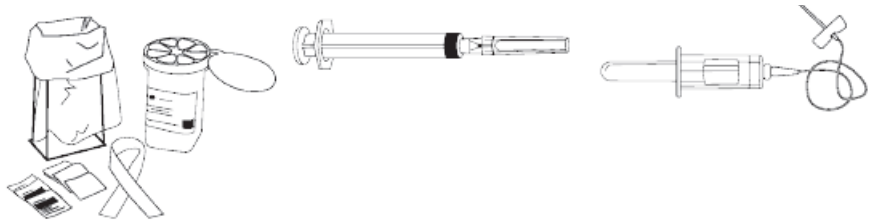
The HemoCue WBC DIFF System is calibrated at the factory and no further calibration is needed.

The software (HemoCue® WBC DIFF Analyzer has an internal quality control (QC) – the self-test. Every time the analyzer is turned on, it will automatically verify the performance of the analyzer. If the test fails, an error code will be displayed. See Operating Manual [1] for troubleshooting guide.

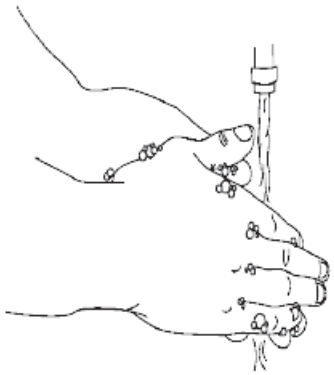
References

[1] HemoCue® WBC DIFF System. Operating Manual. Version 190425.
[2] Osei-Bimpong A, Jury C, McLean R, Lewis SM. Point-of-care method for total white cell count: an evaluation of the HemoCue WBC device. Int J Lab Hematol. 2009;31(6):657-64.
[3] Jönsson I, Nilsson M, Wahlström S, Johnsson E, Jonasson-Bjäräng T, Lindberg S. Novel technology for 5-part differentiation of leukocytes Point-of-Care. Abstract. 24th International Symposium at the AACC CPOCT Division October 4-6, 2012, Prague, Czech Republic.
[4] Hambleton K, Connolly CM, Borg C, Davies JH, Jeffers HP, Russell RE, Bafadhel M. Comparison of the peripheral blood eosinophil count using near-patient testing and standard automated laboratory measurement in healthy, asthmatic and COPD subjects. Int J Chron Obstruct Pulmon Dis. 2017;12:2771-2775.
[5] Kur DK, Agersnap N, Holländer NH, Pedersen OBV, Friis-Hansen L. Evaluation of the HemoCue WBC DIFF in leukopenic patient samples. Int J Lab Hematol. 2020;42(3):256-262.

Annex 1: Best practices in phlebotomy (WHO, 2010)

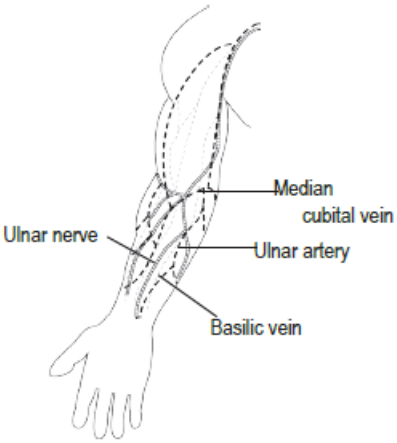


1. Assemble equipment and include needle and syringe or vacuum tube, depending on which is to be used.

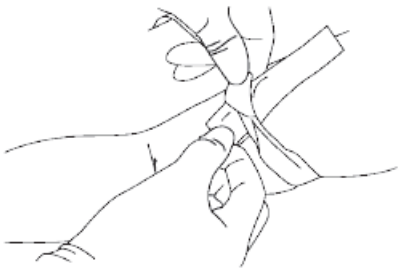


2. Perform hand hygiene (if using soap and water, dry hands with single-use towels).

3. Identify and prepare the patient.

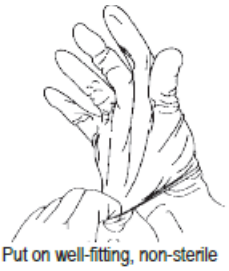


4. Select the site, preferably at the antecubital area (i.e. the bend of the elbow). Warming the arm with a hot pack, or hanging the hand down may make it easier to see the veins. Palpate the area to locate the anatomic landmarks. DO NOT touch the site once alcohol or other antiseptic has been applied.



5. Apply a tourniquet, about 4–5 finger widths above the selected venepuncture site.

6. Ask the patient to form a fist so that the veins are more prominent.



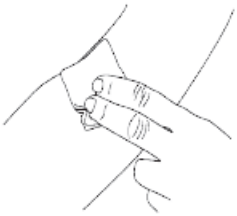
7. Put on well-fitting, non-sterile gloves.

8. Disinfect the site using 70% isopropyl alcohol for 30 seconds and allow to dry completely (30 seconds).

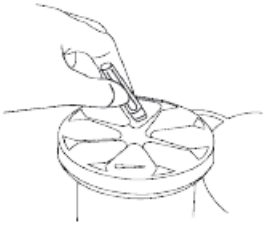
9. Anchor the vein by holding the patient's arm and placing a thumb BELOW the venepuncture site.

10. Enter the vein swiftly at a 30 degree angle.

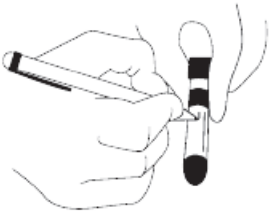
11. Once sufficient blood has been collected, release the tourniquet BEFORE withdrawing the needle.



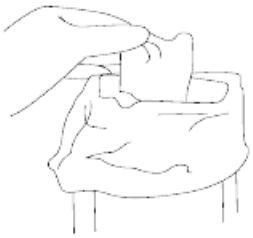
12. Withdraw the needle gently and then give the patient a clean gauze or dry cotton-wool ball to apply to the site with gentle pressure.



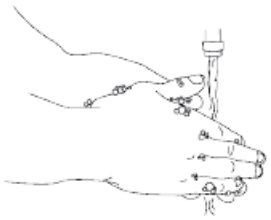
13. Discard the used needle and syringe or blood-sampling device into a puncture-resistant container.



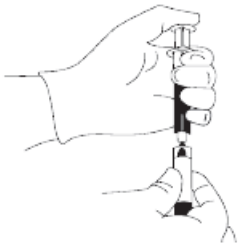
14. Check the label and forms for accuracy.



15. Discard sharps and broken glass into the sharps container. Place items that can drip blood or body fluids into the infectious waste.

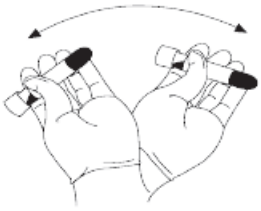


16. Remove gloves and place them in the general waste. Perform hand hygiene. If using soap and water, dry hands with single-use towels.



1. If the tube does not have a rubber stopper, press the plunger in slowly to reduce haemolysis (this is safer than removing the needle).

2. Place the stopper in the tube.



3. Following laboratory instructions, invert the sample gently to mix the additives with the blood before dispatch.

Annex 2: Instructions for hand washing

Duration of the entire procedure: 40-60 seconds

Figure copied from WHO guidelines on hand hygiene in health care (2009).

Annex 3: Datasheet

	First day of sampling (Monday)	Second day of sampling (Friday)
Day of the week and date		
Time-sample		
Duration of sleep		
Coffee/alcohol intake		
Physical activity		
Smoking		
Medication use		
Other remarks		

Sex: ☐ Male ☐ Female

Date of birth:

Appendix N. Collection, pre-processing and storage of urine

1. Long-term study: Collection, pre-processing and storage of urine (source: ECRHSIV)

Introduction

Urine is collected in ECRHSIV for assessment of internal exposure to conservatives and disinfecting agents (BRUsH). The aim is to collect at least 10 ml of urine from each participant and from this to prepare urine samples for long term storage at -80°C. EPHOR do not have funding for analyzing urine samples in the long-term study (e.g. markers of environmental and occupational exposure, cotinine), but the stored urine will be available for EPHOR.

Participants are asked to refrain from eating and drinking for 12 hours prior to urine collection.

Only polypropylene materials should come in contact with urine. Make sure not to use triclosan-containing soap or other triclosan-containing products before handling the urine samples.

Materials needed

Collection:	Atago Urine Specific Gravity Refractometer, PAL 10-S, ATAGO U.S.A., Inc., WA 98005 USA Polypropylene tubes (2 mL) Labels participant ID – sample ID (including date and time of collection) If printed labels are not available: Black ink pen (permanent) or cryomarker Glass pipettes and rubber bulb Disposable gloves (powder-free, nitrile) Biological waste container -Posters with instructions for hand washing (see annex 2 above)
Storage:	- Storage box - Freezer -80 °C

Procedure for collection of urine

Collection of urine

Participants are provided with a wide mouthed sterile container (polypropylene) to collect the urine. Label cup with study ID, time and date of specimen.

- Preferable to collect urine at the same time for each participant –first morning void is optimal. If this is not possible, make sure to register at what time during the day the urine was collected.

Processing and storage of urine at site of collection

Wait at least 15 minutes: do not use material which is over 30°C

1. Measure urine specific gravity in the sample. Record results on sample log-sheet.
    - Use a handheld refractometer (Atago Urine Specific Gravity Refractometer, PAL 10-S, ATAGO U.S.A., Inc., WA 98005 USA). Calibrate refractometer before each use.
    - Shake the urine sample well before measuring urine specific gravity (e.g. 8-10 times by inversion).

2. Pipette urine into 2 ml polypropylene tubes
    - Leave some room between urine and cap. Screw caps on tubes on snugly.
    - Replace the stopper securely. Label the tube.
    - Place sample in freezer – if possible - at -80°C. Store all samples upright in special storage boxes.

Discard empty urine cup appropriately. Remove gloves and wash your hands.

Document urine specimen in logbook: study ID, time and date of specimen, urine specific gravity, number of aliquots/tubes (and size of aliquots if these vary).

NB! Blank/control samples. Field blank specimens should be prepared once a week during the project period. Field blanks can be prepared with deionized water processed using the exact same protocol as the study samples and stored in 1 polypropylene tubes. Field blank specimens will be used in order to exclude contamination at collection and handling of the samples.

2. Short term study: Collection, pre-processing and storage of urine (source: EPHOR WP3)

Introduction

Urine serves as a non-invasive matrix for the assessment of internal exposure to metals and polycyclic aromatic hydrocarbons (PAHs).

Materials needed

Collection:	- Urine collection containers polypropylene - Labels: participant ID – sample ID (including date and time of collection) - Pipettes and tips - 10% nitric acid solution for washing - Posters with instructions for hand washing (see annex 1)
Pre-processing:	- Polypropylene tubes (1.5 mL and 5 mL) (EPHOR) - Labels: participant ID – sample ID (including date and time of collection) - Disposable gloves - Biological waste container
Storage:	- Storage box - Freezer -80 °C (EPHOR)

Collection of urine

Pre-treatment of urine collection containers

The following steps must be followed:

- Ten percent nitric acid solution is put in a tank.
- The urine collection containers are opened and together with the screw caps, completely immersed for at least 3h in the solution (preferably overnight).
- Next, the containers and caps are rinsed three times with purified water.
- The containers and caps are put face down in a clean filter paper to dry (preferably in an oven at 60°C).
- After drying, the containers are closed by us of their screw cap.

Pre-treatment of the containers is not necessary in case:

- the research team has already checked for contamination beforehand and in case they are stored appropriately. This is done by performing blank measurements with purified water in the containers to ensure satisfying levels of metals.
- containers free of metal is used (the laboratory must be contacted before using this option).

Urine collection

Participants are asked to refrain from eating and drinking for 12 hours prior to urine collection.

A pre-treated, labeled container and hermetic bag are distributed to the participant. Work clothes (e.g. overalls) are removed before urine collection. Hands are washed according to the instructions (see annex 2: instructions for hand washing, from 'Collection, pre-processing and storage of blood').

Next, the cap is removed from the container, urine is collected, the container is closed and put inside the hermetic bag. After collection, the bag with container is handed over to the research team.

Pre-processing and storage of urine at site of collection

Aliquots of at least 2 mL are made in polypropylene tubes of 5 mL. Half of them are labeled for metal analysis, the other half for PAH analysis. Empty urine containers are disposed in a biological waste container. The urine samples are stored at -80 °C until shipment to CUT and KUL.

Participants are asked to refrain from eating and drinking for 12 hours prior to urine collection. A pre-treated, labeled container and hermetic bag are distributed to the participant.

<u>Instructions to the participants:</u>

- Work clothes (e.g. overalls) are removed before urine collection.
- Hands are washed according to the instructions (see annex 2: instructions for hand washing, from 'Collection, pre-processing and storage of blood').
- Next, the cap is removed from the container, urine is collected, the container is closed and put inside the hermetic bag.
- After collection, the bag with container is handed over to the research team.

Appendix O. Microbiom/Gingival fluid sampling procedure

Aim

To collect a sample of oral microbiome from gingiva for bacterial DNA extraction.

Supplies

- Sterile paperpoints: Protaper Universal Paper Points F4 and F5 (F4: Ø40 and F5: Ø50)
- 2 ml Microtubes safelock Biopur (2 tubes)
- Sterile mirror and sterile tweezers
- Sterile gloves
- Surgical face mask

Sterile Procedure: Right hand must be sterile through the whole procedure. Left hand semi-sterile.

Sample collection

Use the mirror to hold the lip(s) aside/apart. The sterile paperpoint is introduced (with sterile tweezer or hand with sterile glove) in the gingival area, between the gingiva and the tooth, alongside the tooth, hold there for 5 seconds.
One paperpoint is inserted at each of the following places, in both the upper and lower mouth:

1. Between the two frontal teeth
2. Left frontal tooth, lateral side
3. Right frontal tooth, lateral side
4. Left molar number 6, facing molar number 5*
5. Right molar number 6, facing molar number 5*

*If molar number 6 is missing, use molar 5

Paperpoints are then placed in sterile tubes (to be opened and closed with the left hand);
one tube for the 5 samples (paperpoints) from upper mouth,
one tube for the 5 samples (paperpoints) from lower jaw,
marked separately.

Storage

Freeze the sample directly at -20°C followed by storage at -80°C

Appendix P. Saliva sampling and storage procedure

Aim

To collect salvia for bacterial and individual DNA extraction.

Supplies

Sterile supplies to include (but not limited to) tubes, pipets, pipet tips, and buffer

- Sterile gloves (for handling of samples in the lab)
- Falcon, 50 mL sterile conical polypropylene tubes for collection
- 2 ml Microtubes safelock Biopur (2-3 tubes)
- Sterile phosphate buffered saline (PBS)

Sample collection

The subject should hold the sterile collection tube (Falcon) himself/herself. Subject is asked to swallow and sit with the head down to allow saliva to collect and then drain off the lower lip into the Falcon tube. The subject should avoid touching the top of the tube and inside the cork. The collection period may take several minutes or may be repeated in order to collect larger volumes of saliva (aim for a minimum of 2 mL).

Laboratory procedure

1. Immediately mix the sample with equal volume of sterile PBS buffer (but a minimum of 300 µL)
2. Mix saliva and buffer in the tube by inversions 8-10 times
3. Transfer sample to 2 ml tubes (minimum 2 tubes)
4. Label the tube with the subjects' id, date and sample type
5. Freeze the samples directly at -20°C followed by storage at -80°C

Field blanks: Store tubes with PBS buffer (without samples) - which has been handled by the same procedure as in the preparation for sample storage. If the samples are collected in Falcon tubes and then transferred to smaller tubes, do the same procedure for the field blank samples (but with PBS buffer only in the tube). Prepare 1 field blank sample once or twice a month for the duration of the project period. The field blanks can be used to determine external contamination.

Appendix Q Baseline and screening questionnaire (short-term study)

This screening questionnaire for the WP6 Short-term study is under development. It will be supplemented and revised after finalizing the feasibility study in WP1 and pilot study in WP3.

#	QUESTION	POSSIBLE VALUES	REFERENCE
	GENERAL QUESTIONS		
1	Participant ID	Numeric	
2	Operator ID	Numeric	
3	Date	Date	
4a	ZIP code to your home (Please can you write your post code here?)	Numeric	WP3 Pilot (EC4 Screening)
4b	ZIP code to your work place	Numeric	
5	Pollen count (if available)	Numeric	
	CONTRAINDICATIONS, AIRWAY INFECTIONS AND ENVIRONMENTAL EXPOSURE		
6	Have you had a heart attack during **the last 3 months**?	No	EC4
		Yes	
7	Have you had surgury related to the eye, stomach- or breastregion during **the last 3 months**?	No	EC4
		Yes	
8	Are you pregnant?	No	EC4
		Yes	
8a	If YES, are you in pregnancy **week 29 or more**?	No	EC4
		Yes	
9	Are you currently under treatment for tuberculosis or other airway infection?	No	EC4
		Yes	

#	QUESTION	POSSIBLE VALUES	REFERENCE
10	Have you had any airway infection (e.g. a cold) during the **last 3 weeks**?	No Yes	EC4
10a	If YES, are you recovered from the airway infection?	No Yes	EC4
10b	If YES, how many days since you felt recovered?	Number of days	EC4
11	Have you smoked during the last hour?	No Yes	EC4
12	Consumption of food containing nicotine (to be specified)	No Yes	Pilot study WP3 (smoking)
13	Consumption of smoked food	No Yes	Pilot study WP3 (PAH)
14	Have you taken one or more tablets for your allergy the last 7 days?	No Yes	EC4
15	Are you taking any medication for your asthma or COPD?	No Yes	EC4
15a	If YES, write the name of your medication and note date and time for last dose in the table below.	Table 1 (see sheet "TABLES")	EC4
	OCCUPATIONAL EXPOSURE		
16	What is your present occupation?	String	
17	What year did you start in your last work engagement?	Year	
18	Work hours per day?	Numeric	
19	Exposed hours per day?	Date	EPHOCAS
20	When did you finnish your last work shift? (Time elapsed since last occupational exposure)	Time	
21	Do you experience respiratory symptoms related to your job situation?	No Yes	SIC Bergen Q

21a	If YES, please indicate when the respiratory symptoms appear after exposure at work	Acute	
		Later	
		Nocturnal	
		Acute and late	
		Acute and nocturnal	
		Other	SIC Bergen Q
21b	If YES, is there any improvement in symptoms during	Weekends	
		Vacations	
		Wekkend and vacations	
		No improvement weekend/vacations	SIC Bergen Q
22	When did your symptoms start?	Year	SIC Q
23	How long did it take from starting at your present work place (occupational exposure) and the onset of work-related respiratory symptoms?	Months	EPHOCAS (modified)
24	How long were you working with symptoms? (Duration of work exposure after the onset of asthma symptoms)	Months	EPHOCAS (modified)

Appendix R. App questions "How am I" for short-term study

Source: EPHOR WP1, version 19.06.2021

Daily questions EPHOR WP6

Introduction

1. Do you work regular hours (day, evening or night) or on a shift pattern?
    a. Regular hours day
    b. Regular hours evening
    c. Regular hours night
    d. Shift pattern no night shifts
    e. Shift pattern with night shifts

2. In this study, the app will inform you twice daily that a short questionnaire must be completed in the app, once in the morning and once in the evening. Please indicate for each day what would be an appropriate time to be notified by the app that a questionnaire is open:
    a. Morning: [HH] : [MM 0/15/30/45]
    b. Evening: [HH] : [MM 0/15/30/45]

    ➔ Please be aware that the morning questionnaire is open 6am-12noon and the evening questionnaire is open 7pm-11pm.

Morning

1. At what time did you go to sleep in the last 24 hours?
    a. 15 minute options, [HH] : [MM 0/15/30/45]

2. At what time did you get up in the last 24 hours?
    a. 15 minute options, [HH] : [MM 0/15/30/45]

3. How would you rate your sleep quality (referring to your last sleep)?
    a. Very good
    b. Fairly good
    c. Fairly bad
    d. Very bad

4. How often were you woken by your asthma during the night?
    a. VAS: 0 not woken at all, 100 awake all night

5. How bad were your asthma symptoms when you woke up this morning?
    a. VAS: 0 no symptoms , 100 very severe symptoms

Evening

6. Did you wear any personal protective equipment today?
    a. Yes, but only due to COVID-19 precautions
    b. Yes
    c. No

7. What personal protective equipment did you wear?
    a. Body protection
    b. Hand protection
    c. Foot protection
    d. Eye and face protection
    e. Head protection
    f. Hearing protection
    g. Respiratory protection

8. When did you leave home today to go to work?
    [HH] : [MM 0/15/30/45]

9. At what time did you arrive at work?
    a. [HH] : [MM 0/15/30/45]

10. Which mode of transport did you use to get to work?
    a. Car
    b. Bus
    c. Train
    d. Subway
    e. Tram
    f. Cycle
    g. Walk
    h. Other

11. When did you leave work today to go home?
    a. [HH] : [MM 0/15/30/45]

12. At what time did you arrive at home?
    a. [HH] : [MM 0/15/30/45]

13. Which mode of transport did you use to get home?
    a. Car
    b. Bus
    c. Train
    d. Subway
    e. Tram
    f. Cycle
    g. Walk
    h. Other

14. How many alcoholic beverages did you consume in the last 24 hours?
    a. numbers_____

15. Did you exercise in the last 24 hours?
    a. Yes
    b. No

16. What intensity level of exercise did you do and how long for? (Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal, for example, running; Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal, for example, brisk walking or light cycling; Light activities require the least amount of effort such as walking slowly or light garden/house work.)
    a. Vigorous/HH:MM [0/15/30/45]
    b. Moderate/HH:MM [0/15/30/45]
    c. Light/HH:MM [0/15/30/45]

17. In general, how was your mood today?
    a. VAS: 0 very negative, 100 very positive

18. In general, how relaxed or tensed were you today?
    a. VAS: 0 very tense, 100 very relaxed

19. In general, how energetic did you feel today?
    a. VAS: 0 very tired, 100 very energized

20. How much were nose symptoms bothering you today?
    a. VAS: 0 not at all bothersome, 100 extremely bothersome

21. How much were eye symptoms bothering you today?
    a. VAS: 0 not at all bothersome, 100 extremely bothersome

22. How much were asthma symptoms bothering you today?

a. VAS: 0 not at all bothersome, 100 extremely bothersome

23. How limited were you in your activities today because of your asthma?
    a. VAS: 0 not limited at all, 100 totally limited

24. How much shortness of breath did you experience today?
    a. VAS: 0 none, 100 a very great deal

25. How much of the time did you wheeze today?
    a. VAS: 0 not at all, 100 all the time

26. Please score how many puffs of short acting bronchodilator you have used in the past 24 hours.

    0 none
    1 1–2 puffs
    2 3–4 puffs
    3 5–8 puffs
    4 9–12 puffs
    5 13–16 puffs
    6 More than 16 puffs

Appendix S. Passive samplers of chemical exposure

Source: WP1, Feasibility study

Passive sampler

Passive samplers are easy-to-use sampling devices for measurement of chemicals: volatile and semi-volatile organic compounds (VOCs and SVOCs), in the air. The uptake of chemicals is mainly based on molecular diffusion. The passive samplers for EPHOR are specially designed and consist of a small aluminium tube filled with an adsorbent (Tenax TA) to capture VOC. A piece of silicone hose is attached around the tube, which captures SVOCs. Around the silicone hose there is protective mesh cover to avoid contact with clothes and skin (hands).

The following analytes will be analyzed:

- Semi-volatile organic compounds (PDMS, passive sampling, GC-MSMS):
  - Polycyclic aromatic hydrocarbons (PAH)
  - Organochlorine pesticides (OCPs)
  - Organophosphate ester (OPE)
  - Pyrethroids
- Volatile organic compounds (Tenax, passive sampling, ATD-GCMS):
  - Volatile phthalates
  - Volatile PAHs
  - Volatile OPEs
  - Organic acid anhydrids
  - Aldehydes/ketones
  - Benzene, toluene, etylbenzene and xylene (BTEX)
  - Phenols
  - Solvents
  - Alcohol/disinfectants
  - Microbial volatile organic compounds

The analytical methods are established methods and sample preparation and analysis will be done in the TNO lab. Thus no detailed description of these steps are included in this SOP for fieldworkers.

Procedure

Preparation

- The passive samplers are sent to the centers in an airtight tube with a screw cap. An envelope for the return will be enclosed. The tube is provided with a label and unique code that is linked to the participant.
- Before application, the samplers have to be kept at room temperature in the airtight tube.

Application

- Upon application the sampler is removed from the tube.
- The passive sampler with the size of a pen (12 x 100mm), is equipped with a clip and a buckle to attach the sampler to clothes. Alternatively, the sampler can also be attached to a necklace.
- The sampler should be placed in the breathing zone at chest- or shoulder height (figure 4).
- The sampler is worn for five days continuously and must be attached to the out layer of clothing (not covered by other clothes).
    - Outside, the sampler must be attached to the jacket.
    - While sleeping, the sampler must be placed in the bedroom (on the bedside table).
    - While showering the sampler must be place in the bathroom outside the shower cubicle.
    - While sporting, if it's not possible to attached the sampler on the clothes it can be placed in the vicinity of the participant.

At the end of the sampling period

- After use (which is expected to be ~5 work days) the sampler will be put immediately back in the same airtight tube with the screw cap attached.
- The tube is provided with a label where the participant's code and the period of sampling period must be entered.
- After that, the tube will be returned to TNO in the enclosed envelope.

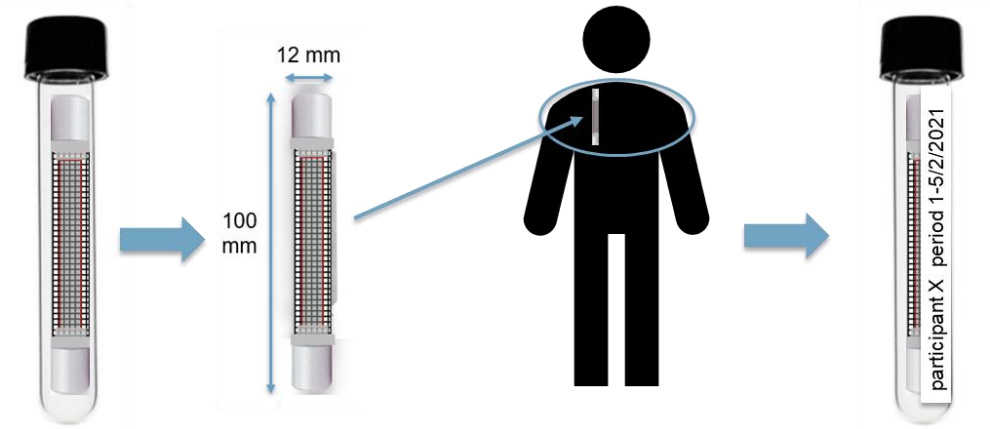


Figure 1. The pen sized passive sampler in its transport tube (left and right) and exposed to ambient air (second from right). The sampler must be worn in the breathing zone as indicated in this figure (second from left).

Appendix T. The sensor system in the short-term study

Source: WP1, Feasibility study

The sensor system being developed by WP1 will include four devices: sensor box, activity sensor, passive sampler and heart rate sensor. A feasibility study is ongoing, and based on feedback from the participating centers the protocol will be adjusted. The feasibility study is focusing on performance of the sensor (technical issues) and feasibility of all methods, accompanying SOPs, and the overall base fieldwork protocol. The devices is briefly described below.

Sensor box

The sensor box will measure physical exposures (EPHOR, developed at VTEC, Eindhoven, the Netherlands). The EPHOR sensor is shown in figure 1 and comprise the following:

- Sensor box with the dimensions 73 x 54 x 20 mm
- EPHOR sensor charging cable, USB-A to USB-micro
- EPHOR sensor mount for personal measurements
- Gateway (VTEC)
- Gateway charger, power plug to USB-C (Pi4)

The sensor box has a measurement frequency of 1 time/minute, and measures:

- Particulate matter (PMs), including PM_1, $PM_{2.5}$, PM_{10}
- Ambient temperature (°C)
- Relative humidity (%)
- Sound (dB(A))
- Light intensity (lux)
- UV intensity (W/cm^2)

The sensor box will be worn using an “across the chest harness” on the outside of clothing with the box held by the harness and located in the center of the chest. Alternatively, the sensor box may be worn around the waist on a belt.The sensor box has an expected battery life of 10 hours and will take 2.5 hours to charge. The sensor box will be worn uncovered on the participants body, whenever possible or kept in their direct vicinity.

In addition to the sensor box, a gateway unit will be provided (the sensor and gateway are shown figure 1). The purpose of the gateway is to download the collected data from the sensor box via Bluetooth and store it. The gateway is a small box, that needs to be continuously plugged in (in order to maintain power) and connected to the internet via a LAN cable in the participants home.

The time this sensor will be worn for is TBD. The data from the sensor box sampler needs to be downloaded/charged each day (during sleep). At the end of the study period, data will be downloaded from the gateway onto protected computers at the specific study site.

Figure 1 Sensor box (right) and gateway (left).

Activity sensor

The activity sensor (Ax3, developed by Axitivity) has an accelerometer that will measure activity as well as sleep (figure 2). The battery life is 30 days. This activity sensor will be worn around the wrist. The time this sensor will be worn for is TBD (and still TBD how charging of this device works, as they will need to have the device on them to get accurate information on sleep). There is a "gateway" that the devices will be charged and communicate with to download participant data to.

Figure 2. Activity tracker.

Heart rate monitor

There will be a heart rate monitor (Polar H10 Heart rate sensor, manufactured by POLAR) will be worn around the sternum to measure heart rate (figure 3). The heart rate monitor has an approximate battery life of 300 hours or more, and a weight of 21 g (connector) and 39g (strap). The data from the HR monitor will be downloaded (how- still TBD in collaboration with WP1).

Figure 3. Heart rate monitor.

Appendix U. Electrostatic Dust Fall Collector (EDC)

Method Description of the Electrostatic Dust Fall Collector (EDC)

Gitte Juel Holst 26th of April 2011, updated 04th of January 2021 Vivi Schlünssen; 10th of May R. Bertelsen

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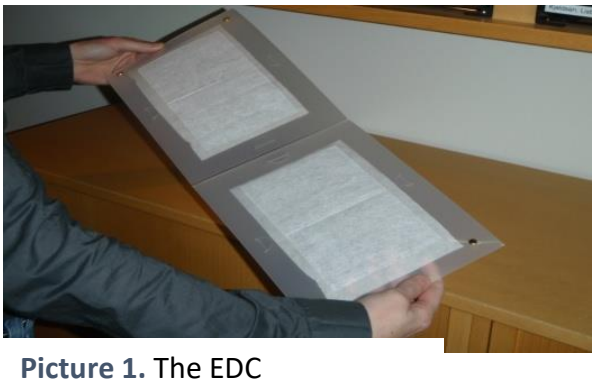
Aim

The electrostatic dust fall collector (EDC) aims to collect airborne dust settling on the surface. The EDC is in particular suitable for exposure assessment in residential and occupational epidemiological studies due to the low-cost, efficient and manageable nature of the EDC (1-3).

Principle

The EDC combines several passive airborne dust sampling methods both the pizzabox/dustfall collector method (4) and the electrostatic cloth sampling method (5). The EDC consists of a custom-fabricated polypropylene sampler that has electrostatic cloths attached to it to provide a sampling surface. Airborne dust settles on this surface and is captured by the electrostatic properties of the cloth. Extraction of dust components from the electrostatic properties allows for assessments of long-term microbial associated molecular patterns (MAMP's), microbial abundance and diversity, and allergen exposures. The EDC sampler has been validated within both urban and rural homes, with respect to reproducibility within homes and between two sampling periods, showing reproducibility over time equivalent to reservoir dust analyses.

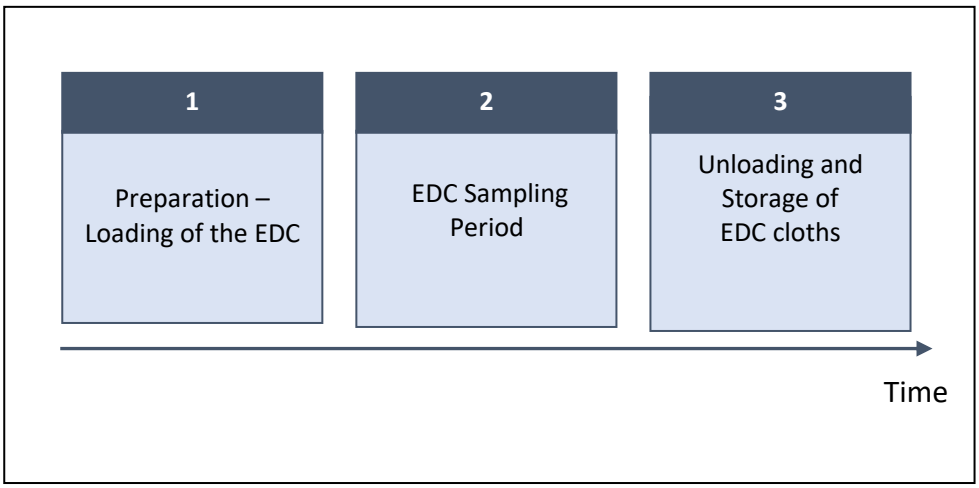


Picture 1. The EDC

Process of airborne dust sampling with the EDC

The process of airborne dust sampling with the EDC is illustrated in figure 1 and will be described in the following.

Figure 1. Process of airborne dust sampling with the EDC



1. Preparation – Loading of the EDC

Equipment and material

- Sampler
- 2 electrostatic cloths (Stofwisdoekjes, Zeeman) per sampler, packed in aluminium foil, steril-
  ised at 200°C for at least 4 hours
- Sticker to mark the sampler
- 2 pair of tweezers
- Lab gloves
- Lab coat
- <70% ethanol
- Paper tissues
- 4 split pens per sampler, packed in aluminium foil, sterilised at 200°C for at least 4 hours
- 1 paperclip per sampler

Procedure

Wear lab coat and lab gloves during preparation. Clean workplace and hands thoroughly with ethanol and collect all needed materials. Clean sampler by wiping it with ethanol soaked paper tissues. After sterilizing the electrostatic cloths at 200°C for at least 4 hours in aluminium foil open the package with the cloths. Take the cloth out of the package by using sterile tweezers. Between each step the tweezers are bathed in ethanol and directed through a flame. Cloths may only be touched with a clean tweezer. Position the cloths in sampler, so that it fits under the frame. Fold the frame over the cloths and tighten it with the split pens in the according holes. It is easier to prepare each side of the folder separately. The finished sampler can now be sealed with a paperclip in each corner. A manual for assembly of the EDC is found in appendix 1.

2. EDC sampling period

The EDC is handed out either by instructed personnel or send to the participants by post.

Instructions to participants

The participants may open the EDC carefully without touching the cloths. The participants must be instructed to install the EDC in their bedroom or in their work place location 150 cm above the floor on a flat area where to expect as little air turbulence as possible e.g. away from windows, doors, ventilation and heaters. The participants must be instructed to leave the sampler open for 14 days without touching and removing the sampler. Date and time of opening and placing the sampler, exact height, and unintended events such as a cat has been sitting on the sampler or the cloths have been touched are reported in the feedback form. After 14 days the sampler must be closed slowly and carefully to ensure that the sampled dust is not disturbed. Seal the sampler with a paperclip and ship back in pre-paid enveloped addressed to the laboratory/field station. An instruction on how to place the passive sampler is found in appendix 2.

3. Unloading and storage of EDC cloths

Equipment and material

- The envelop with the exposed EDC sampler
- 2 pair of tweezers
- Lab gloves
- Lab coat
- >70% ethanol
- Paper tissues
- 2 zipped bags pr. sample depending on the number of clothes on the EDC
- 2-4 pre-labled stickers or a permanent marker to mark the minigrip pags
- At least 2 storage boxes, depending on the number of clothes on the EDC

Unloading procedure

Considering potential bacterial growth, it is highly recommended to unpack and store the cloths as soon as possible and no later than two weeks after receiving the EDCs in the laboratory/field station. Wear lab coat and lab gloves during the unloading procedure. Handle one wipe at the time. Start by labelling the zipped plastic bags with ECRHSIV, date, study centre ID, and participation id. Clean workplace and hands thoroughly with ethanol. Only handle one EDC at the time. Remove the EDC and any accompanying material from the participant's envelope. Remove the safety pins from each corner of the EDC. Open the safety cuttings. Place the labelled plastic bags next to the sampler. Sterilize the two pair of tweezers by putting them in ethanol and directed through a flame. Each wipe is removed by the sampler using a pair of sterile tweezers. It is folded twice and then placed carefully on the zipped, pre-labelled bag. The bag is sealed and placed in the storage box. Clean the working area and hands with ethanol soaked paper tissues between each sampler. An instruction on how to unload and store the passive sampler is found in appendix 3.

Storage

The two cloths must be distributed in two different storage boxes in order to facilitate the individual use of the cloths. About 40 to 60 cloths can fit in one box. The box should be labelled (ECRHS IV, box 1A, 1B, 2A 2B etc.) to allow identification of parallel project

samples. In order to prevent bacterial growth long-term storage should always take place either at -20 or -80 ^{0}C. To reduce dramatically endotoxin activity repeated freeze-and-thaw cycles are not recommended.

References

(1) Noss I, Doekes G, Sander I, Heederik DJ, Thorne PS, Wouters IM. Passive airborne dust sampling with the electrostatic dustfall collector: optimization of storage and extraction procedures for endotoxin and glucan measurement. Ann Occup Hyg 2010 Aug;54(6):651-658.

(2) Noss I, Wouters IM, Bezemer G, Metwali N, Sander I, Raulf-Heimsoth M, et al. beta-(1,3)-Glucan exposure assessment by passive airborne dust sampling and new sensitive immunoassays. Appl Environ Microbiol 2010 Feb;76(4):1158-1167.

(3) Noss I, Wouters IM, Visser M, Heederik DJ, Thorne PS, Brunekreef B, et al. Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. Appl Environ Microbiol 2008 Sep;74(18):5621-5627.

(4) Wurtz H, Sigsgaard T, Valbjorn O, Doekes G, Meyer HW. The dustfall collector--a simple passive tool for long-term collection of airborne dust: a project under the Danish Mould in Buildings program (DAMIB). Indoor Air 2005;15 Suppl 9:33-40.

(5) Thorne PS, Metwali N, Avol E, McConnell RS. Surface sampling for endotoxin assessment using electrostatic wiping cloths. Ann Occup Hyg 2005 Jul;49(5):401-406.

Appendix 1. Manual for the assembly of the Electrostatic dust fall collector (EDC)

Material:

- Sampler
- 2 electrostatic cloths (Stofwisdoekjes, Zeeman) per sampler, packed in aluminium foil, sterilised at 200°C for at least 4h
- Sticker to mark the Sampler
- 2 pair of tweezers
- Lab gloves
- Lab coat
- <70% Ethanol
- Paper tissues
- 4 Split pens per sampler, packed in aluminium foil, sterilised at 200°C for at least 4h
- 1 Paperclip per Sampler

Adding the electrostatic cloths:

1. Clean workplace and hands (with gloves!) thoroughly with Ethanol and collect all needed materials (Sampler, electrostatic cloths, paper tissues, tweezers and Splitpens). Wear lab coat and lab gloves from now on to prevent contaminations.

Figure 1

2. Clean sampler (fig 2) by wiping it with Ethanol soaked paper tissues.

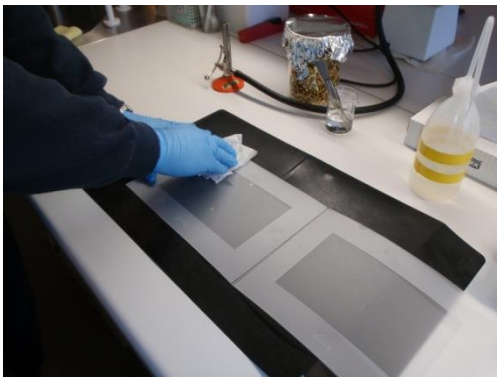


Figure 2

3. Take electrostatic cloths packed in aluminium foil, sterilised at 200°C for at least 4h and open the package (fig 3+4)

Figure 3

Figure 4

4. Take the cloth out of the package by using sterile tweezers (fig 7). Between each step the tweezers are bathed in Ethanol and directed through a flame (fig 5 6). Cloths are only to be touched with a clean tweezer.

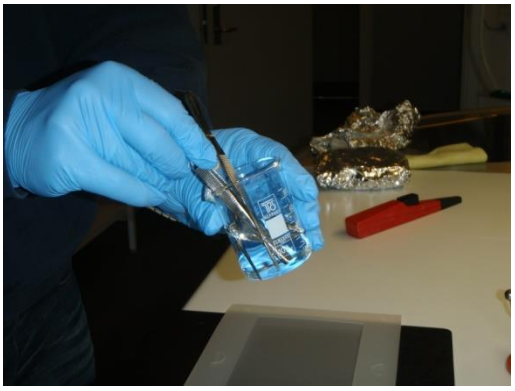


Figure 5

Figure 6

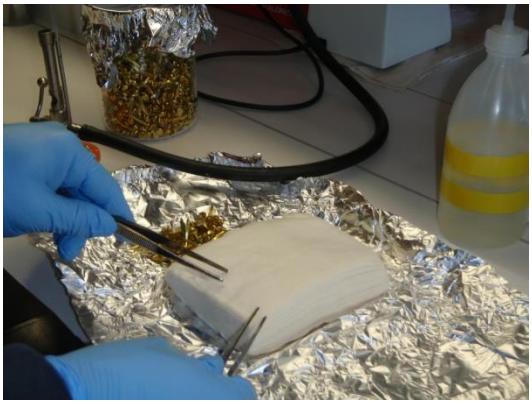


Figure 7

5. Position cloths in sampler, so that it fits under the frame (fig 8 9).

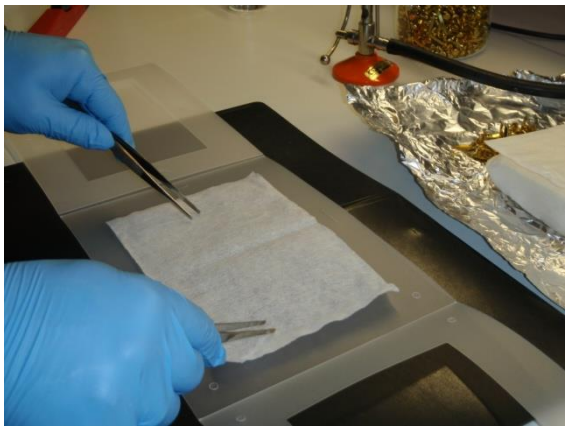


Figure 8

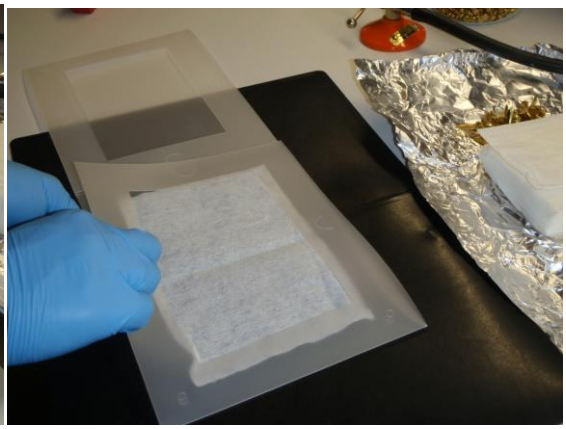


Figure 9

6. Fold the frame over the cloths and tighten it with the split pens in the according holes (fig11 12). It is easier to prepare each side of the folder separately.

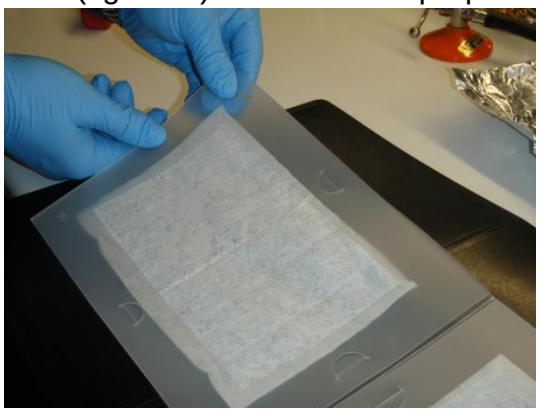


Figure 10

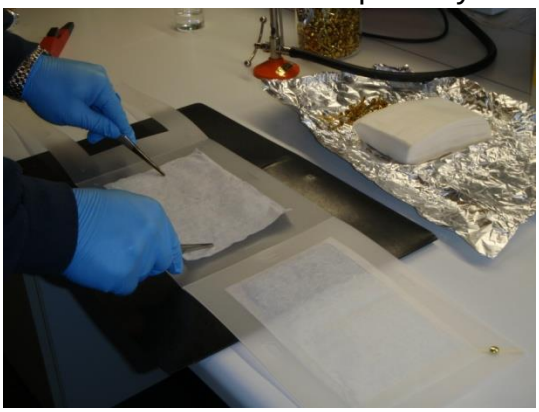


Figure 11

7. The finished sampler (fig 12+13) can now be sealed with a paperclick (fig 14+15) and send by mail to the participants of the study.

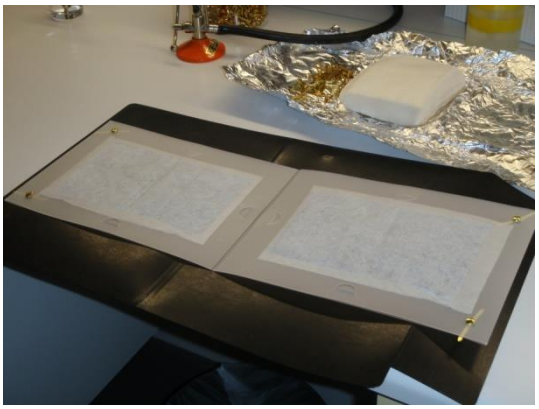


Figure 12

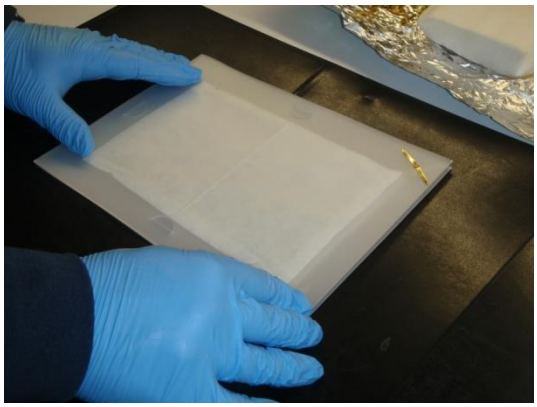


Figure 13

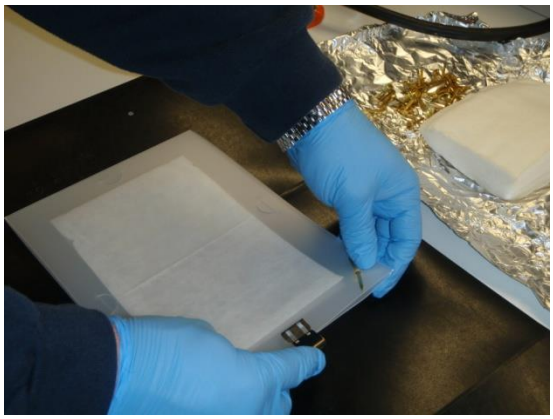


Figure 14

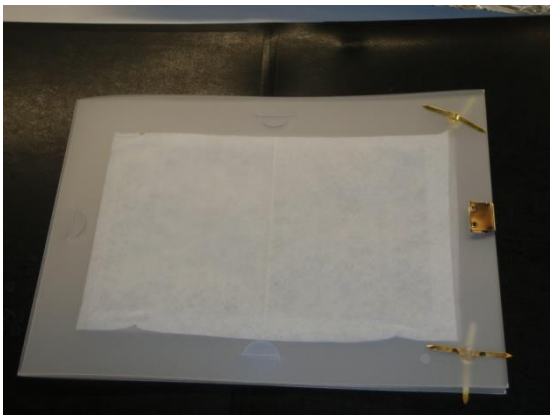


Figure 15

Appendix 2. How to handle the EDC

At first take the sampler carefully out of the envelope. From now on only touch the sampler on the plastic. Open it carefully by sliding of the paperclip. Unfold the folder.

Only touch the sampler on the plastic parts and never touch the electrostatic cloths.

Position the sampler on a place at least 150 cm above ground and with as little tendency to sudden air disturbances as possible (i.e. not close by doors or windows). Sampler should not be placed in the kitchen, nearby heating systems, air conditioners, TV, or computers.

Leave sampler in for 14 days and do not touch or move it during that time! Please write down: Date and time of opening and placing the folder, exact height from the floor where sampler was placed. If irregularities occurred, such as a cat sitting on it or something fell on it, please also write that down.

After 14 days each single folder should be closed slowly and carefully so that the sampled dust is not disturbed. Seal the folder with a paperclip and touch only the plastic parts!
The sealed folder can now be placed in the envelope and send back to the Laboratory.

Thank you for taking part in this study!

Information sheet on the passive dust sampler

This form is used together with the passive dust sampler

Regarding handling and placing of the passive sampler, please follow the instructions in the enclosed manual. After placing the sampler, please write the actual date and the precise height the sampler is placed in. After 14 days, please close the sampler and write the date. Please also state if unintended events have occurred (e.g. a cat has stepped on the sampler; something fell on the sampler etc). Put the sampler and this feedback form in the pre-paid return envelope, and mail it.

Information on the participant:

1. Name_____ 2. Study ECRHS ID: _____

3. Address: _____

4. Do you have any potted plants in the bedroom? [1]❑ *Yes* [2]❑ *No*

Information on the housing:

5. What type of housing do you live in:

[1]❑ Detached house
[2]❑ Farmhouse without livestock
[3]❑ Farmhouse with livestock
[4]❑ Multifamily house/ terraced house
[5]❑ Apartment
[6]❑ Other _____

6. Size of your house: _____m^2 7. Size of your bedroom: _____ m^2

Information on the dust sampler <u>in the bedroom</u>:

8. The date of opening and placing the sampler: |__|__|/|__||__|/|__||__|

9. At which height the sampler is placed: _____ cm above the floor

10. The date of closing the sampler: |__|__|/|__||__|/|__||__|

11. Unintended events: ……
……
……

Additional questions about the passive dust sampler placed in the bedroom

12. At which floor is the bedroom situated? [1]❑ Basement [2]❑ Ground floor [3]❑ Higher

13. Which flooring does the bedroom have:

[1]❑ Wall to wall carpet [2]❑ Lacquered wooden floor [3]❑ Unlacquered wooden floor [4]❑ Other flooring

What kind of surface does the bedroom walls have? (Mark each line with a cross)

14. Wallpaper [1]❑ *Yes* [2]❑ *No*
15. Painted wallpaper [1]❑ *Yes* [2]❑ *No*
16. Painted fibreglass texture [1]❑ *Yes* [2]❑ *No*
17. Other, what kind_____ [1]❑ *Yes* [2]❑ *No*

18. What kind of windows does the bedroom have?

[1]❑ Single layer [2]❑ Single layer with an extra glazing in front [3]❑ Double glazing [4]❑ Triple glazing

[5]❑ Other

What type of heating is used in the bedroom? (Mark each line with a cross)

19. Hot water radiators [1]❑ *Yes* [2]❑ *No*
20. Electric radiators [1]❑ *Yes* [2]❑ *No*
21. Floor heating [1]❑ *Yes* [2]❑ *No*
22. Woodstove/fireplace [1]❑ *Yes* [2]❑ *No*
23. Other, what kind? _____ [1]❑ *Yes* [2]❑ *No*

What type of ventilation is in the bedroom? (Mark each line with a cross)

24. Sliding windows [1]❑ *Yes* [2]❑ *No*
25. A vent or a grid in the ceiling [1]❑ *Yes* [2]❑ *No*
26. A vent or a grid in external walls or windows [1]❑ *Yes* [2]❑ *No*
27. Exhaust fan in the ceiling or external walls [1]❑ *Yes* [2]❑ *No*
28. Other, what kind?_____ [1]❑ *Yes* [2]❑ *No*

29. Have you noticed visible mould/fungi on the floor, walls or on the ceiling in the bedroom?

[1]❑ *Yes* [2]❑ *No*

30. Have you noticed visible damp patches on the floor, walls or the ceiling in the bedroom?

[1]❑ *Yes* [2]❑ *No*

31. Do you suspect any problems with dampness and/or mould/fungi which is not visible inside in the house i.e. inside the floor, walls and ceilings? [1]❑ *Yes* [2]❑ *No*

Has there been any flooding or other kind of water damage in: (Mark each line with a cross)

32. The bedroom? [1]❑ *Yes* [2]❑ *No* [3]❑ *Don't know*

33. Other places in the house? [1]❑ *Yes* [2]❑ *No* [3]❑ *Don't know*

Thank you for participating!

Appendix V. Lung function with hand-held spirometer Vitalograph micro

1. Aim of procedure

The procedure aims to measure daily (change in) lung function over a work week, measured as FEV_1 and FVC (without bronchodilator).

Each spirometry session must be carried out according to the SOP described in the Section below. For details, see User Training Manual for Vitalograph micro Model 6300. Trained staff should instruct the participant in using the handheld spirometer and ensure that the measurements are valid (see section 7.3.1-2 below).

All study staff who undertake the lung function tests are asked to read this document and to be familiar with its contents and that of this SOP. A copy of this document should be kept with each spirometer in case questions arise during testing.

2. Smoking, diet, physical activity and medication use prior to testing

Participants should refrain from smoking, big meals or heavy physical activity at least one hour before the investigation for one hour prior to testing.

Medication use. In order to provide a valid lung function assessment, participants should be asked to refrain from taking short-acting beta-2 agonist (SABA) (e.g., albuterol or salbutamol) at least 4 hours before doing the manoeuvre.

3. About the spirometer

Spirometry during the follow-up week in the short-term study will be conducted using the **Vitalograph micro model 6300 spirometer** (Vitalograph Ltd, UK) (picture 1). The device is a handheld, portable spirometer that measures participants' respiratory parameters including FVC, FEV_1, and FEV_6. It measures flow and volume by ultra-sound transit time. The Vitalograph micro complies with ATS spirometry standards.

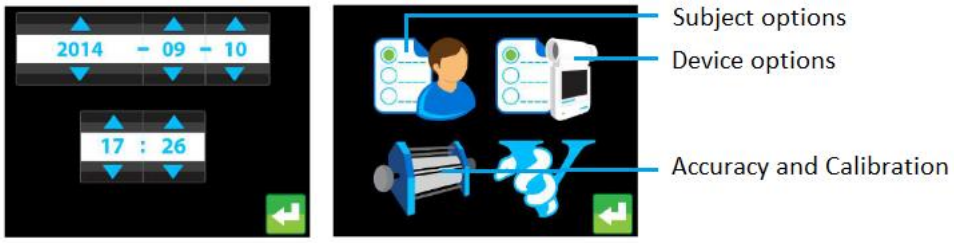
Picture 1 A Vitalograph micro model 6300.

The Vitalograph micro has been designed to need no calibration. The instrument can however develop faults and we request that a calibration check to be carried out on Monday morning as well as an accuracy check Friday morning when returned back to the test personnel. Instructions for performing the calibration check and accuracy check are in the Vitalograph micro User manual, but a short description is given below (see section 9).

4. Setting up the Vitalograph micro

Always check that the Vitalograph micro configuration settings are set to the study parameters and install the Vitalograph micro software in the English language version. A nominated person responsible for configuration of the Vitalograph micro™ should be designated at each clinical site.

Make sure that the correct time and date are given on the Setup screen (picture 2A). To access the configuration screen (picture 2B), press .



Picture 2 Configuration and access to "Set up screen" for Vitalograph micro.

4.1 Vitalograph micro configuration settings for the device

Select "Device option"  to figure the parameters given below.

4.1.1 General Settings

Variable		EPHOR-setting		Variable	
	% Predicted		X	Z-score	
	Sound On	X		Sound Off	
	User Passcode – On/Locked		X	User Passcode – Off/Unlocked	
	Temperature	Default: 23 °C			

4.1.2 Parameters to be displayed on the results screen

Choose the Parameters option  to select what parameters you want to display on screen (max. 8). Use the left/right arrows to navigate between the screens. The following has to be included:

Parameter	**Definition**	
FEV_1	Forced expiratory volume after 1 second (L)	The amount of air that a person exhales during the first second of a forced expiratory manoeuvre.
FEV_6	Forced expiratory volume after 6 second (L)	The amount of air that a person exhales during the six first seconds of a forced expiratory manoeuvre.
FVC	Forced vital capacity (L)	The total volume of air exhaled in a forced expiratory manoeuvre.
FEV1/FVC	Ratio FEV_1 of FVC	

5. Preparing for spirometry

5.1 Location and equipment

During a spirometry manoeuvre there is a small risk that the participant may faint and hurt him/herself while falling. **Participants must therefore perform the manoeuvres in the seated position, in a chair with arms but without wheels.** Spirometry testing ideally should be performed in a private, temperature-controlled room. For safety, the participant must be ask to be seated during the procedure.

5.1.1 Equipment

Vitalograph micro spirometer
Clean mouthpieces (Spirettes)
Spare AA batteries (XX batteries)
Calibration syringe & syringe adapter (1 and 3 liters)
Sink for hand washing, soap and hand towels

Containers to collect: used BVFs
Box of tissues
Alcohol wipes
Disposal bin
Clinical gloves
Chair with arms/without wheels
Drinking water and cups/glasses

The participant will bring home the following equipment:
Vitalograph micro spirometer
Mouthpiece – bacterial viral filter (BVF) (5 filters)
Spare AA batteries (4 batteries)
Alcohol wipes
Plastic bag

5.2 Infection control

Standard precautions for airborne infection control must be applied, including cleaning and disinfection of the spirometer between participants (see section 11 for procedure). Staff and participants should wash their hands before the start of the test.

6. Performing the spirometry (procedure)

6.1 Creating a New Subject

1. Press the On/Off switch on the front face of the instrument to turn the device on.

2. To create a new subject (participant) touch the icon  and enter subject details by touching the fields on the screen, including age, height, sex and weight. Press 'Enter' after each entry.

6.2 Testing of lung function

1. Fit a disposable BVF™ to the device (flowhead).

2. To start the spirometry session: Select the "FVC"-test option  from the Main menu.

3. Perform spirometry testing when the icon  is visible. This indicates that the Vitalograph micro unit is ready to accept a blow. Take care not to block the mouthpiece with the tongue or teeth during testing. The results may be viewed as either a Volume/time (V/t) or Flow/Volume (F/V) graph by pressing the graph button on the side of the test screen. Select the F/V mode.
4. Have participant assume correct posture (seated). The participant is asked to perform an ordinary manoeuvre including FEV_6 (6 seconds). The device will give a signal (sound) when the manoeuvre is satisfactory.
6. Repeat the blow at least three times to obtain good test quality.
7. The results summary on the bottom of the screen shows the FEV_6 and FEV_1 of the last blow.
8. The number of usable blows and bad blow indicator "(!)" are shown in a separate box next to the last test FEV_1 and FEV_6. There will also be an audible sound at the end of test to indicate a bad blow. The participant has to perform 3 usable FEV_6-blows.
9. After performing the tests press the enter button to exit the **Test** screen. This brings you back to the **Main Menu**.

6.3 Instructions to participants

A good instruction with the participant will improve the quality of the test. Explain that the purpose of the test is to take some measurements to check on the health of the lungs. Emphasize that, although the procedure does not hurt, in order to get useful and valid results he/she must breathe out as hard and as fast and for as long as is possible when told to do so, and will need to repeat the procedure three times at least.

Allow the participant to insert the BVF onto the spirometer. If using a BVF, be careful to ensure that the smiley on the mouthpiece is turned against the participant in the upright position.

6.3.1 Instruction to participants

After instructing the participant about the procedure for pulmonary function testing the following procedures should be followed.

1. Take in as deep a breath as possible with a pause of 1 s at TLC
2. When his/her lungs are totally full, quickly position the mouthpiece – close lips around the mouthpiece
3. Exhale maximally (hard and as fast as possible) until no more air can be expelled while maintaining an upright sitting position
4. Blow out smoothly **without re-breathing.**
5. Continue exhaling for at least 6 seconds
6. Throughout they should remain erect and not bend forward
7. All participants to have a minimum of 3 **attempts** at a full FVC/FEV_6 manoeuvre.

To assist the participant – technicians should give a vigorous demonstration in which they

- demonstrate the correct positioning of the mouthpiece
- take a deep breath and emphasize the full depth of inhalation
- demonstrate a dramatic blast out as fast as possible.

6.3.2 Problems with lung function testing

Many factors will result in error, including hesitation or false starts, cough, variable effort, glottis closure, early termination and leaks. When errors do occur, review them with the participant before proceeding with additional manoeuvres. You may wish to repeat a demonstration manoeuvre. Demonstrate the correct placement of the mouthpiece, emphasize the maximum depth of inhalation, and then blast out the air. If the participant tries again and the reproducibility criteria are not met, continue the test as needed (up to a total of 8 manoeuvres), assuming that the participant is able to continue.

When errors occur, review common errors with the participant before proceeding with additional manoeuvres.

7. Reporting, storing of data and data management

7.1 Reports, printing and transfer of data

After the last lung function measurement a pdf-report must be printed and stored from the Vitalograph micro. This is done by connecting the spirometer to a PC running the **Vitalograph Reports** application.

1. To produce PDF reports from the micro, ensure that the software is installed on the PC you wish to report to by running the Vitalograph Reports CD supplied with the micro and following the on-screen instructions.

2. The micro can be connected to the PC either using the USB cable supplied with the device, or for the Bluetooth version a Bluetooth dongle may be used. Ensure that Vitalograph reports is open and the micro is switched on and in the home screen.

3. Centres will be required to have a PC compatible with the Vitalograph Reports application/software.

4. There are no method for exporting the report to a csv. Hence, PDFs from each participant must be combined in Adobe Acrobat and sent in a pseudo-anonymous way to the coordinating center in Aarhus (see below)

Versions of Vitalograph Micro. All centres will be equipped with the same software throughout the period of the study – centres should NOT upgrade during the period of data collection.

7.2 Storing results

The Vitalograph micro has the capacity to store 750 subject entries with the corresponding session data. It is possible to perform up to 20 blows per session, however **only a maximum of the best 3 blows will be stored with each session.**

In EPHOR the Vitalograph micro is intended to be used as a temporary storage device for one participant at a time. It is important that the data belonging to the participant is deleted **after** making sure that the data and the pdf-report has been transferred and stored.

All databases will be regularly merged with the master database at the co-ordinating centre. Hence, in addition to being stored on the centers storage site for the study, the pdf reports must be sent to the coordinating center in Aarhus, Denmark (PI EPHOR WP6 professor Vivi Schlünssen vs@ph.au.dk) where a quality control will be performed (see section 10).

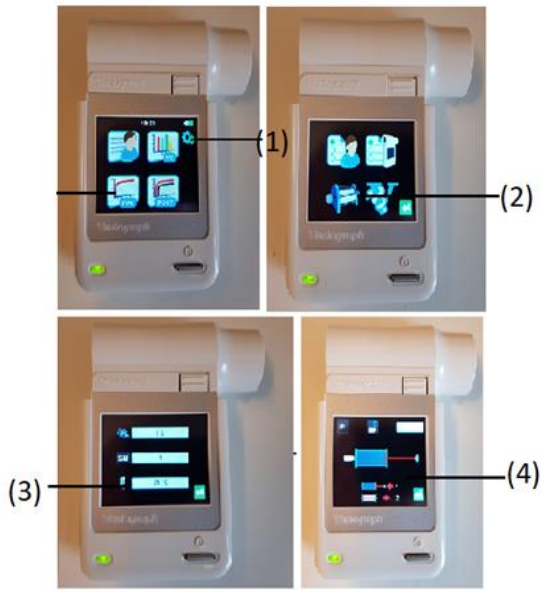
8. Calibration or verification of accuracy

All spirometry standards recommend checking the accuracy of lung function measuring devices at least daily with a 3-L syringe to validate that the instrument is measuring accurately.

For EPHOR we ask you to calibrate the device with syringes of both 1 and 3 liters prior to use (Monday morning). The accuracy of lung function measurements must also be carried out when spirometer is handed in at end of follow-up (Friday morning). The calibration syringes and adapters should always be stored next to the spirometer so that the temperature between them is similar. Completed calibration procedures must be logged. If the volume measured during an accuracy check deviate more than 3% this must also be noted.

8.1 Procedure

1. Select the Configuration button on top right corner of the main menu screen (picture 3, (1))
2. Select the Accuracy/Calibration icon (2)
3. Enter the Syringe volume, reference and ambient temperature (3). Pump air through the flowhead to bring it to ambient temperature.
4. Press the “Enter” key to bring you into the Accuracy Check screen and follow the on-screen instructions (4)



Picture 3 Procedure for calibration and accuracy checks.

5. The Accuracy Check result is shown in % in the top right corner of the screen. If it is reproducible and within 3% a green tick pass icon will be shown. Pressing the Enter key will return you to the main menu.
6. If the Accuracy Check result is outside 3% the error icon will be shown. Pressing the enter key proceeds to the Calibration Update routine to update the Calibration.
7. The Calibration Update screen will show the volume (L) on the top left corner of the screen, next to the number of strokes.
8. The procedure is the same as for the Accuracy Check.
9. Accuracy must also be checked: 1) if the flowhead or device has been dropped, 2) if a new flowhead has been fitted (see user manual 7.8), and 3) after cleaning or disassembling spirometer for any reason.

9. Quality control

Data management including quality control measures for all lung function measurements (pdf-reports with spirometric values and curves) will be performed centrally headed by researchers with extensive experience from former ECHRS and Constances study waves. All material will be assessed by two EPHOR-researchers with experience with evaluating spirometric curves.

We ask each center to provide the coordinating center in Aarhus, Denmark with material from 10 participants at a time so that the Spirometry Curves arising from the testing can be reviewed. Explicit instruction will be provided to each centre at the time for the transfer of pseudo-anonymised data and a brief report will be provided to each centre so that the instruction to the participants doing the lung function testing can be adjusted.

10. Cleaning

10.1 Cleaning and Low Level Disinfecting the Vitalograph micro

The device must be cleaned and disinfected between participants. In general, the parts of the Vitalograph micro that make up the flowhead, which comes into contact with subjects being tested, require **low level disinfection**.

The body of the device may be **cleaned** with an alcohol wipe (70% isopropyl alcohol). For the screen, lightly wipe the surface with cotton pad or other soft material (not in a circular motion).

10.2 Disassembling and Cleaning of the Fleisch Flowhead

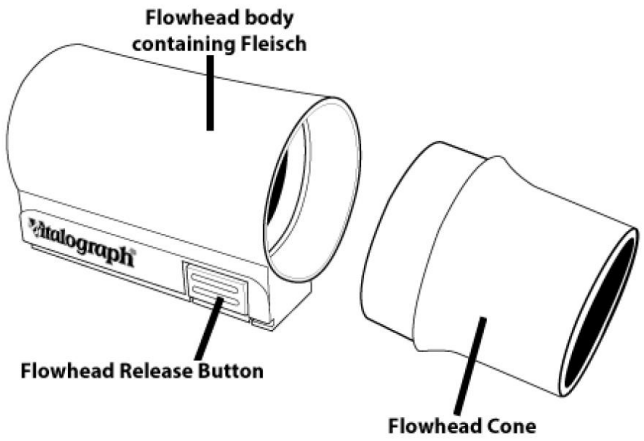
1. Hold the device body firmly in your left hand.
2. Hold the flowhead with your right hand, at the same time press and hold the button firmly on the front of the fleisch flowhead.
3. Slide the flowhead away from the device from left to right.
4. Remove the **flowhead cone** from the **flowhead** (picture 4), by twisting and pulling it away from the flowhead.
5. To clean the flowhead body swill vigorously in warm soapy water. Do not attempt to “rub” or “scrub” at capillaries.
6. To clean the flowhead cone wash in warm soapy water. Rub surface to remove any visible soiling.
7. Examine all parts to ensure they are visibly clean. If not visibly clean repeat the cleaning process.
8. Rinse all parts in distilled water.
9. Leave to dry completely before reassembling. Drying the fleisch element may require placing it in a warm place overnight. A drying cabinet is ideal.

10.3 Reassembling and Low Level Disinfection of the Fleisch Flowhead

1. Examine the fleisch element to ensure that no liquid or particles remain in the holes, grooves or pressure tappings.

2. Examine the rubber grommets at the top of the device to ensure no liquids or particles remain in the holes. Also ensure the grommets are not damaged.
3. Fit the flowhead cone to the flowhead.
4. Slide the flowhead into the grooves in the top cover. The Vitalograph logo and button on the flowhead should be on the same face as the LCD when assembled.
5. Wipe all external surfaces of the flowhead with a 70% isopropyl alcohol impregnated cloth.

A calibration check must carried out following reassembly.



Picture 4 Flowhead components.

11. Service and other procedures

Beyond battery replacement and the calibration/accuracy check, the spirometer requires no maintenance. No service should be performed on the spirometer except by manufacturer-authorised personnel.

For procedures on service and fault finding, see User manual.

Appendix W. Collection, pre-processing and storage of exhaled breath
(source: EPHOR WP3)

Introduction

A panel of volatile compounds on breath related to mechanisms such as oxidative stress and inflammation makes up the core of the analysis, while discovery analysis will be performed on the collected data to identify new potential compounds which correlate occupational exposures. The volatile compounds on breath will be measured and identified by high resolution GC-MS.

Materials needed

Collection:	- CASPER portable clean air supply o CASPER system (see figure 1) o Air filter o Air supply tubing - Shelf trolley - ReCIVA breath sampler and accessories o ReCIVA breath sampler (see figure 2) o Head strap o Dymo barcode label printer o Barcode labels (linking participant ID, date and time of sampling to the participant's electronic case report form) o Barcode scanner o Laptop with supplied software - Breath biopsy kit (one per patient per sample) o Single-use mask assembly o Single-use sorbent tubes x4 - Electronic case report form (eCRF): the software on the supplied laptop has an in-built eCRF which requires several basic questions to be answered (see table 1)
Pre-processing:	Not required
Storage:	Refrigerator (4-8 °C)

Collection of exhaled breath

Participants are asked to refrain from eating, drinking (except for water), smoking or brushing their teeth for 1 hour prior to sampling.

The participant places the ReCIVA breath sampler mask over their nose and mouth. This is held in place by a head strap.

The study subject is then required to breath normally into the mask. The system will calibrate and adjust to match the breathing pattern of the individual, consequently, they will not experience increased resistance whilst breathing into the mask. The breath sampling procedure typically takes 10-15 minutes, this includes the time required to fill-in the basic eCRF.

Throughout the procedure, the subject's breathing is continuously monitored by the operator of the system via inbuilt CO_2 and pressure sensors. The procedure can be interrupted or aborted at any time by both the operator and the subject. A

fter sampling has taken place, a barcode on the sorbent tube assembly is scanned using the supplied barcode scanner. This automatically inputs the sorbent tube assembly ID into the participant's eCRF. The supplied barcode label printer is then used to print 3 identical barcodes encoding the patient ID and date and time of sampling. These barcodes are to be used in the shipment of both the exhaled breath and respiratory droplets samples. (Retain one label to attach to the packaged respiratory droplets device.)

Pre-processing and storage of exhaled breath at site of collection

Pre-processing is not required.

After collection, the sorbent tubes (exhaled breath) are stored in a refrigerator at 4-8°C until shipment to Owlstone Medical for analysis within 2-3 weeks of collection.

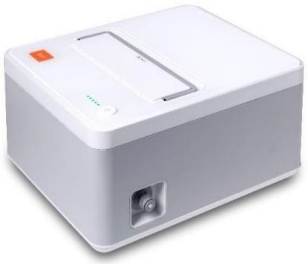


Figure 1. CASPER system

Figure 2. ReCIVA breath sampler

Stage	Question	Format
Pre-sampling	Patient ID	Numeric
Pre-sampling	Age	Years
Pre-sampling	Gender	Male/female
Pre-sampling	Study Center	Free text, pre-populated
Pre-sampling	Height and weight	Height in cm, weight in kg
Pre-sampling	Last meal	(Free text) + hours since
Pre-sampling	Last drink	(Free text) + hours since
Pre-sampling	Time since brushing teeth	Does not brush/hours since
Pre-sampling	Occupational vapour exposure	Yes/no + details (free text)
Pre-sampling	Current symptoms	Short of breath (yes/no), fever (yes/no), halitosis present (yes/no), cough (yes/no), cold (yes/no)
Pre-sampling	Time since last cigarette	Does not smoke/hours since
Pre-sampling	Time since last e-cigarette	Does not use/hours since
Pre-sampling	Last e-cigarette flavour	Free text
Pre-sampling	Location	Free text
Pre-sampling	Operator	Free text
Pre-sampling	ReCIVA serial number	Numeric
Pre-sampling	Casper serial number	Numeric
Pre-sampling	Air filter serial number	Numeric
Pre-sampling	Collect type	Drop down - numeric, dependent on study configuration
Post-sampling	Patient comfort level	Very comfortable/somewhat comfortable/somewhat uncomfortable/very uncomfortable
Post-sampling	Patient breathed preferentially via	Drop down - mouth/nose/both/don't know
Post-sampling	Technical or patient feedback about sampling	Free text

Table 1. Electronic case report form (eCRF)

Appendix X. Collection, pre-processing and storage of exhaled respiratory droplets (source: EPHOR WP3)

Introduction

A panel of volatile and non-volatile compounds on breath related to mechanisms such as oxidative stress and inflammation makes up the core of the analysis, while discovery analysis will be performed on the collected data to identify new potential compounds which correlate occupational exposures. Non-volatile breath targets captured can be analyzed using LC-MS and/or ELISA.

The protocol for exhaled breath aerosols is currently under development and is likely to be subject to change (ref. EPHOR WP3).

Materials needed

Collection:	-Single-use exhaled respiratory droplets collection device -Dymo barcode label printer -Barcode scanner -Barcode labels (linking participant ID, date and time of sampling to the participant's electronic case report form) -Laptop with supplied software -Electronic case report form (eCRF): the software on the supplied laptop has an in-built eCRF which requires several basic questions to be answered (see table 1)
Pre-processing:	Not required
Storage:	Freezer (-80 °C)

Collection of exhaled breath aerosols

Participants are asked to refrain from eating, drinking (except for water), smoking or brushing their teeth for 1 hour prior to sampling.

1. The device is labeled.

2. The plastic protective cover is removed from the sampling device.

3. The caps are removed from the sampling device

4. The participant takes a deep breath before exhaling into the sampling device, through the large opening. This is then repeated 12 times before putting the caps back on.

Pre-processing and storage of respiratory droplets at site of collection

Pre-processing is not required. After collection, the single-use respiratory droplets collection device should be stored at -80 °C.

Table 1. Electronic case report form (eCRF)

Stage	Question	Format
Pre-sampling	Patient ID	Numeric
Pre-sampling	Age	Years
Pre-sampling	Gender	Male/female
Pre-sampling	Study Center	Free text, pre-populated
Pre-sampling	Height and weight	Height in cm, weight in kg
Pre-sampling	Last meal	(Free text) + hours since
Pre-sampling	Last drink	(Free text) + hours since
Pre-sampling	Time since brushing teeth	Does not brush/hours since
Pre-sampling	Occupational vapour exposure	Yes/no + details (free text)
Pre-sampling	Current symptoms	Short of breath (yes/no), fever (yes/no), halitosis present (yes/no), cough (yes/no), cold (yes/no)
Pre-sampling	Time since last cigarette	Does not smoke/hours since
Pre-sampling	Time since last e-cigarette	Does not use/hours since
Pre-sampling	Last e-cigarette flavour	Free text
Pre-sampling	Location	Free text
Pre-sampling	Operator	Free text
Pre-sampling	ReCIVA serial number	Numeric
Pre-sampling	Casper serial number	Numeric
Pre-sampling	Air filter serial number	Numeric
Pre-sampling	Collect type	Drop down - numeric, dependent on study configuration
Post-sampling	Patient comfort level	Very comfortable/somewhat comfortable/somewhat uncomfortable/very uncomfortable
Post-sampling	Patient breathed preferentially via	Drop down - mouth/nose/both/don't know
Post-sampling	Technical or patient feedback about sampling	Free text

Appendix Y. Collection, pre-processing and storage of exhaled breath condensate (EPHOR WP3, KU Leuven, latest version 17.03.2021)

Introduction

cfDNA methylation and proteomics will be studied in exhaled breath condensate samples. By using exhaled breath condensate, an alternative matrix for blood is evaluated.

Note: A protocol for collection, pre-processing, storage and analysis of exhaled breath condensate for assessment of cfDNA methylation and proteomics, will be developed during the course of the project and made available after validation (ref. WP3). The protocol given below (Leese and Jones, Health and safety Laboratory, 2020) created for the HBM4EU, is used as a starting point for the pilot study.

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[2]

Authors and Acknowledgements

Lead authors:

This document has been developed by Dr Elizabeth Leese (PhD) and Kate Jones from the Health & Safety Laboratory (HSL).

Contributions were received from: Holger Koch (IPA) and Karen Galea (IOM).

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1 Introduction

According to IARC, hexavalent chromium (Cr(VI)) compounds are classified as carcinogenic to humans (Group I) whereas trivalent chromium (Cr(III)) is an essential element.

In established biomonitoring (urinary total chromium), Cr(VI) exposure cannot be specifically determined because Cr(VI) compounds are reduced to Cr(III) in the body before being eliminated in urine. As exposures reduce due to increased demands from regulators, urinary total chromium methods will become less useful as it will be difficult to separate harmful Cr(VI) exposure from normal dietary Cr(III) exposure. In light of this, more specific biomarkers are being investigated; namely, Cr(VI) in exhaled breath condensate and in red blood cells (where it is trapped and isolated from Cr(III) as only Cr(VI) can enter the red blood cells but in the end it is not CrVI that is analyzed). This SOP covers the collection of exhaled breath condensate (EBC) samples for the analysis of Cr(VI); it is also possible to measure Cr(III) and possibly other metals in samples collected using this procedure.

2 Exhaled Breath Condensate (EBC)

EBC is a biological fluid which consists mainly of water vapour but also of small droplets of airway lining fluid from within the bronchial and alveoli regions of the lungs. These droplets of airway lining fluid contain an unknown fraction of both volatile and non-volatile substances but also environmental and occupational contaminants.

Inhalation is one of the primary routes of occupational exposure. It is thought that EBC might be a useful biological monitoring matrix when the current biological monitoring methods using traditional biological matrices such as urine or blood are not possible, or where the interpretation of elemental species in these matrices is difficult, for example hexavalent and trivalent chromium. Advancing the investigation of EBC may further the understanding of inhalation exposures and how elements behave and reside in the lungs.

The collection and analysis of EBC samples in this project is to further this understanding by gaining a more accurate picture of Cr(VI) exposure by correlating chromium species in EBC samples against industrial hygiene samples (for example personal air samples) in addition to urinary total chromium measurements.

The collection of EBC samples is an non-invasive technique and does not cause an inflammatory response itself. The collection of EBC results in low sample volumes and will take approximately 15 minutes to collect an adequate volume of sample (1-2 mL). Collection of EBC also involes the subsequent step of complexation with an EDTA solution to stabilise the hexavalent and trivalent chromium species immediately after collection.

The volume of EBC collected can vary from one individual to the next. EBC volume is directly correlated to tidal volume and minute/ventiliation volume. Tidal volume refers to the volume of air displaced in the lungs between normal inhalation and exhalation. Minute/ventilitation volume refers to the amount of gas inhaled or exhaled from an individuals lungs in one minute. Individuals with higher minute/ventiliation volume and/or higher tidal volume will produce more EBC[1-3]. This variation in EBC sample volume means the concentration of Cr(VI) will also vary. As there is currently no proposed volume correction marker (such as creatinine for urine) it is therefore suggested that EBC results must be reported in µg/L per volume of EBC collected.

2.1 EBC collection devices

All EBC collection devices are based on a freezing cooling chamber, to cool and condense the exhaled breath. This must consist of an inert material for the surface of the condensing cooling chamber such as glass, aluminium or Teflon as recommended by the American Thoracic Society/European Respiratory Society Task Force[1]. The effectiveness of EBC collection depends on the volume of breath passing into the condenser, the condensing surface area and the temperature gradient of the exhaled air to the cooling chamber[1].

A small number of commercial EBC devices are available to purchase for the collection of EBC. However, due to the differences in collection devices (for example the EBC collection temperature) only the TurboDECCS (Medivac SRL, Parma, Italy) system can be used to collect EBC samples as part of this project.

The TurboDECCS which stands for 'Transportable Unit for Research of Biomarkers Obtained from Disposable Exhaled Condensate Collections Systems', is a portable self contained thermoelectric peltier cooling device. An external power source is required to operate the Turbo, whose function is to cool the EBC collection tube (which is inserted into the cooling unit of the Turbo). The collection tube connects to the disposable EBC sampling kit comprising of a mouthpiece connected to a one-way aspiration valve with a saliva trap (DECCS). (See Figure 1 in section 2.4.1) The temperature for

the cooling chamber is -5ºC (this is also the default temperature setting of the TurboDECCS so adjustment should not be necessary)..

A study by Goldoni et al[4] based on EBC collection volume, biomarker levels and collection variability determined the optimum EBC collection temperature of the TurboDECCS to be -5ºC. Published studies using the TurboDECCS which focus on elemental concentrations in EBC also maintained this collection temperature of approximately 5ºC[5-8].

It is not known how the differences in temperature and humidity of inspired air will affect the collection of EBC. A study by McCafferty et al[9] reported reduced EBC volume when individuals were inhaling cooler and drier air. For this project, EBC samples will be collected indoors in a standard office environment.

The volume of EBC collected can vary greatly however, 15 minutes of tidal breathing will collect on average between 1 – 2 mL of EBC. Several studies support the lack of correlation between EBC volume and gender, age, fitness, smoking status and lung status[2,3,9].

2.2 Complexation of EBC samples

Maintaining the stability and integrity of both Cr(VI) and Cr(III) within any sample matrix can be challenging, as the stability of both species is pH dependent. Generally Cr(VI) is stable in alkaline conditions whilst Cr(III) is stable in acidic conditions and forms Cr(III) hydroxide compounds in weak acidic conditions.

EBC is slightly acidic, with samples checked in earlier work at HSL having a pH between pH 6 and pH 6.5. Stability studies at HSL have shown that EBC samples analysed within 24 hours of collection which contain Cr(III) will elute the Cr(III) at two different retention times when not complexed with EDTA. It is HSL's thought that that the two Cr(III) peaks could be Cr(III) and the product of its slow conversion to a Cr(III) hydroxide.

Secondly as EBC samples and the EDTA solution are both slightly acidic, any Cr(VI) in the EBC samples will begin to slowly convert to Cr(III) if the EDTA solution is not adjusted to pH 8.

Therefore, to maintain the integrity of both chromium species it is impotant that each EBC sample is complexed with an EDTA solution immediately after collection.

A solution of 0.5 mM EDTA is made in water (using ultrapure deionised water – 18.2 MΩhm cm) and adjusted to pH 8 with 10% v/v ammonia solution. The EDTA complexes with Cr(III) and the adjustment of the pH to pH 8 stabilises Cr(VI).

To ensure that the same batch of EDTA solution prepared is used for both complexation of the EBC samples and for preparing standards and control material for the speciation analysis of those samples, a 2L EDTA solution is prepared. 1L can be taken to site to dilute with the EBC samples and the remaining 1L is used for the speciation analysis. The solution is kept at room temperature. Long term stability is unknown, as the solution was made fresh for each set of site visits/analysis in previous studies.

2.3 Standardisation of EBC collection

Although every volunteer will be asked to produce an EBC sample over 15 minutes, this will result in variable volumes of EBC. The variability is due to a result of the amount of air displaced in the lungs during normal inhalation and exhalation known as tidal volume and the amount of gas inhaled or exhaled from the lungs in one minute, known as minute of ventiliation volume. In addition, the droplets of airway lining fluid will be considerably diluted by the condensed water vapour in each

EBC sample, and this dilution will also vary considerably between volunteers. Unfortunately, unlike a urine sample where, for example, creatinine content can be measured to correct for dilution, there has been no such dilution marker proposed for EBC. Until a suitable biomarker of dilution correction can be found it is advised that volume (or weight) of EBC produced by each volunteer is recorded and that results are reported per volume of EBC. Due to the high content of water vapour in an EBC sample, 1 mL of EBC sample will weigh 1 g.

To help standardise the collection of EBC samples further, it is advised the volunteer rinse their mouth with water prior to providing a sample (drinking a cup of water is acceptable). This helps to remove any accumulated food and/or saliva from the mouth, helping to avoid any contamination of the EBC samples.

In addition, for the pre working week EBC samples, it is important that the volunteer has performed no practices or duties where Cr(VI) may occur.

2.4 Collection requirements

2.4.1 Equipment

1. Only the EBC collection device known as a TurboDECCS with disposable EBC sampling kits made of an inert material can be used. The temperature of the TurboDECCS should be at -5ºC which is its default setting.
2. A suitable room away from the primary site of exposure/workshop floor (for example, office, meeting room, first aid/nurses room) with a operational plug socket (to power the EBC collection device) and a table and chair. For the comfort of the volunteer providing the EBC sample, a seated position with the collection device placed in front of them on a table is the most suitable.
3. Labels / or permanent marker pen
4. Nitrile disposable gloves or other suitable gloves
5. Secondary sample tubes with caps suitable for trace metal analysis. For example 15 mL - 50 mL polypropylene screw cap Sarstedt
6. Pipettes & tips (a 100 – 1000 µL, 20 – 200 µL and 500 – 5000 µL pipettes are the most suitable).
7. 0.5 mM EDTA solution adjusted to pH 8 with 10% v/v ammonia solution.
8. Drinking water & cups.

2.4.2 Collection

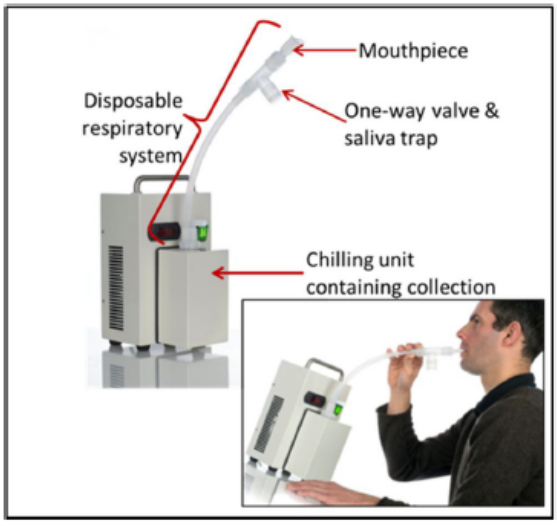
- A suitable room to collect EBC samples should already have been decided away from the primary site of exposure/workshop floor, for example office, meeting room or nurse/first aid room. To standardise the environmental conditions as much as possible ensure the room is within general office space conditions, for example a room temperature between 18-25ºC.

  Plug the Turbo into a suitable power source that enables it to stand on a table where the EBC collection will take place. From room temperature it can take up to 20 minutes for the cooling chamber to reach -5ºC.

- Wearing gloves unwrap and assemble a DECCS sampling kit and insert the EBC sample collection tube into the cooling chamber.

- The DECCS sampling kits are individually wrapped and sealed and so will need opening and assembling according to the instructions available within each bag. For hygiene and potential contamination reasons, each sampling kit is opened and assembled prior to each volunteer and not collectively beforehand.
- For each subsequent EBC sampling kit, ensure the collection tube is inserted in the cooling chamber for at least 5 minutes prior to a volunteer beginning to provide their breath sample to allow the collection tube to cool.

- Ask the volunteer to rinse/wash their mouth out with water (depending on the facilities in the room, the volunteer can rinse their mouth by drinking the water or rinsing and spitting out the water) and then begin providing their breath sample by regular tidal (normal) breathing into the mouth piece for 15 minutes. For the most part a complete seal around the mouthpiece with the mouth and lips must be maintained, however periodic removal will be required to allow any accumulated saliva to be swallowed.
  - It may be advantageous to liken breathing into the mouthpiece to that of breathing with a snorkel or scuba equipment, and to breath through their mouth and not their nose. Remind them to keep their breathing tidal, as heavy or deep breathing may cause them to feel light headed.
- After 15 minutes and the volunteer has ceased providing their sample, remove the entire sampling kit from the Turbo unit, unscrew, cap and label the sample collection tube. Dispose of the remaining sampling kit, for example, in a biohazard bag.
- All samples are to be kept refrigerated at approximately 2-8ºC after collection and complexation, during transportation to the laboratory and once at the laboratory until analysis, DO NOT FREEZE.



Please note that the disposable respiratory system (sampling kit) has been redesigned by medivac to be much shorter. The position of the volunteer to the mouthpiece is now much closer to the chilling unit than depicted in this photograph.

Figure 1. The Turbo-DECCS exhaled breath condensate collection device

⚠ If the Turbo is left without a collection tube inserted into the condensing cooling chamber for too long, the surface of the chamber will begin to form ice, prohibiting another collection tube from being inserted.

⚠ If a tube containing an EBC sample is left in the condensing cooling chamber for too long after the volunteer has ceased breathing into the sampling kit, the sample will begin to freeze. A frozen sample will deteriorate the integrity of Cr(VI).

2.4.3 Complexation with EDTA solution

Each individual will produce a different amount of EBC sample. It is therefore necessary to make a judgement as to what volume of EBC to use in the complexation with the pH adjusted EDTA solution. This is done on-site immediately after collection. Wearing gloves:

- Label a secondary sample tube and aliquot a suitable amount of the EBC sample into this tube. Note how much of the EBC has been transferred to enable accurate calculation of the volume of EBC collected for the reporting of the results.
- Dilute the aliquoted EBC sample 10-fold with the pH adjusted EDTA solution and cap.

An ideal scenario would be to aliquot 1 mL of EBC, so when diluted 10-fold with the EDTA solution it gives a final sample volume of 10 mL (ideally speciation analysis is performed in duplicate, and at least 1.5 mL is required per duplicate. This will leave the remaining complexed sample for any necessary repeats and if managanese & nickel analyses are required).

  - It is very possible a volunteer will produce less than 100 µl of EBC sample. This sample may not be suitable for analysis as a 10-fold dilution may produce an inadequate final volume for speciation analysis (determine minimum analytical volumes needed from your analysing laboratory).

- After complexation, place all EBC samples in a portable refrigeration unit/insulated box with ice pack until the samples arrive back at the analysing laboratory. DO NOT FREEZE.
- A short term storage study at HSL determined that these samples are stable for up to 6 weeks when stored refrigerated. It is not known how long the samples can be stored beyond this before Cr(VI) begins to deteriorate and convert.
- Upon returning to the laboratory, the remaining volume of EBC (uncomplexed) must be weighed and recorded. Each g of EBC correlates to 1 mL of EBC. To this weight, add (in g) the volume of EBC aliquoted for dilution with EDTA to give the original collected weight/volume of sample.

The collection pots are sealed within the sampling kits so cannot be weighed beforehand.

  - Centrifuge all the collection pots (to remove EBC from the side walls)
  - Weigh (g) and record an empty 30mL medicine beaker
  - Transfer the EBC sample to the 30mL medicine beaker and weigh (g) and record again.
  - Transfer back to the original container or another suitable container if retention of uncomplexed sample is required for other assays. Store appropriately for those assays.

2.5 Sample traceability

A standardised convention will be used to assign unique identification codes for all samples collected. The identification code convention is as follows:

Country ID (XX) - Participant ID (XX) - Sample ID (BXX/UXX/EXX/AXX/WXX)

Country ID 'XX' is the country code, using the ISO Alpha-2 country codes for the participating countries[1].

Country	ISO Alpha-2 country codes
Belgium	BE
Finland	FI
France	FR
Italy	IT
Poland	PL
Portugal	PT
The Netherlands	NL
United Kingdom	UK

Participant ID 'XX' is a two-digit running number of participants in each country (e.g. 01 for the first participant recruited, 02 the second and so forth).

Sample ID 'UXX' is one letter (B/U/E/A/W) to identify the type of sample collected, followed by a two-digit identifier (XX) to identify the running number of each type of sample for that worker (e.g. 01 for the first sample, 02 for the second and so forth). The letter code applied for the sample types is as follows:

Type of sample collected	Sample type code
Air	A
Blood	B
Exhaled breath	E
Urine	U
Wipe	W

[1] http://www.nationsonline.org/oneworld/country_code_list.htm

The following scenario is provided to illustrate the application of this convention.

A worker is recruited in The Netherlands. He is the first worker recruited and is providing his first EBC sample. The sample identification code assigned is therefore:

NL- 01-E01

In the event that an air sample is also collected from this same worker, the sample identification code to be assigned would be:

NL- 01-A01

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Appendix I. Constances Cohort Study - Educational groups and job title

72. *Quelle était la catégorie socioprofessionnelle de votre père* ***pendant votre adolescence*** *?*

- ☐ 1 Agriculteur exploitant
- ☐ 2 Artisan, commerçant, chef d'entreprise
- ☐ 3 Cadre, profession intellectuelle supérieure (ingénieur, médecin…)
- ☐ 4 Profession intermédiaire (professeur des écoles, infirmier, assistant social, technicien, contremaître, agent de maîtrise…)
- ☐ 5 Employé (employé de bureau ou de commerce, garde d'enfants, agent de service…)
- ☐ 6 Ouvrier
- ☐ 7 Au foyer, sans profession
- ☐ 8 Autre, précisez :
- ☐ 9 Ne peut pas répondre

73. *Quelle était la catégorie socioprofessionnelle de votre mère* ***pendant votre adolescence*** *?*

- ☐ 1 Agricultrice exploitante ou conjointe sur exploitation
- ☐ 2 Artisan, commerçante, chef d'entreprise ou conjointe collaboratrice
- ☐ 3 Cadre, profession intellectuelle supérieure (ingénieur, médecin…)
- ☐ 4 Profession intermédiaire (professeur des écoles, infirmière, assistante sociale, technicienne, contremaître, agent de maîtrise…)
- ☐ 5 Employée (employée de bureau ou de commerce, garde d'enfants, agent de service…)
- ☐ 6 Ouvrière
- ☐ 7 Au foyer, sans profession
- ☐ 8 Autre, précisez :
- ☐ 9 Ne peut pas répondre

74. *Quel est le diplôme* ***le plus élevé*** *que vous ayez* ***obtenu*** *?*

- ☐ 1 Sans diplôme
- ☐ 2 Certificat de formation générale (CFG), Certificat d'études primaires, Diplôme national du brevet (BEPC ou Brevet des Collèges)
- ☐ 3 Certificat d'aptitude professionnelle (CAP), Brevet d'études professionnelles (BEP)
- ☐ 4 Baccalauréat ou diplôme équivalent [(a)]
- ☐ 5 Bac +2 ou +3 [(b)]
- ☐ 6 Bac +4 [(c)]
- ☐ 7 Bac +5 ou plus [(d)]
- ☐ 8 Autre, précisez :

[(a)] *Exemples : Capacité en droit, Diplôme d'accès aux études universitaires (DAEU), Brevet Professionnel (BP), Brevet de Technicien (BT), Probatoire du Diplôme d'Etudes Comptables Supérieures (DECS)*

[(b)] *Exemples : Brevet de technicien supérieur (BTS), Diplôme universitaire de technologie (DUT), Brevet de Maîtrise (BM), Diplôme des Métiers d'Arts (DMA), Diplôme d'études universitaires générales (DEUG), Diplôme d'études universitaires scientifiques et techniques (DEUST), Licence, Licence professionnelle*

[(c)] *Exemples : Maîtrise, Maîtrise des Sciences et Techniques (MST), Maîtrise des Sciences de Gestion (MSG), Master 1*

[(d)] *Exemples : Diplôme d'études approfondies (DEA), Diplôme d'études supérieures spécialisées (DESS), Master 2, Doctorat, Diplôme de recherche technologique (DRT)*

Appendix II. Constances Cohort Study - Physical activity

II. ACTIVITÉ PHYSIQUE

3. *Dans **votre travail actuel** ou dans **votre dernier emploi si vous ne travaillez pas actuellement**, quel degré d'effort physique vous est-il (était-il) demandé **habituellement** ? (une seule réponse possible)*

- ☐ 1 Travail sédentaire
- ☐ 2 Travail léger : marche, petite manutention (moins de 10 kg)
- ☐ 3 Travail moyen : manutention d'objets assez lourds (entre 10 kg et 25 kg)
- ☐ 4 Travaux de force : manutention lourde (25 kg et plus)
- ☐ 5 N'a jamais travaillé

4. *Au cours des **12 derniers mois**, avez-vous fait **régulièrement** des trajets à pied, à vélo... (pour le travail ou non) ?*

- ☐ 1 Non
- ☐ 2 Oui, moins de 15 minutes par trajet
- ☐ 3 Oui, 15 minutes et plus par trajet

➥ **Si oui** (quel que soit le temps de trajet) :

- Combien de fois par semaine **en moyenne** ? ☐☐
- Depuis combien d'années ?
  - ☐ 1 Moins d'1 an
  - ☐ 2 1 an ou plus
    - ➥ Indiquez depuis combien d'années : ☐☐ année(s)

5. *Au cours des **12 derniers mois**, avez-vous fait **régulièrement** du sport (hors trajets, bricolage, jardinage et ménage) ?*

- ☐ 1 Non
- ☐ 2 Oui, moins de 2 heures par semaine
- ☐ 3 Oui, 2 heures et plus par semaine

➥ **Si oui** (quel que soit le nombre d'heures) :

- Combien de fois par semaine **en moyenne** ? ☐☐
- Depuis combien d'années ?
  - ☐ 1 Moins d'1 an
  - ☐ 2 1 an ou plus
    - ➥ Indiquez depuis combien d'années : ☐☐ année(s)

6. *Au cours des **12 derniers mois**, avez-vous fait **régulièrement** des travaux de bricolage, jardinage ou ménage ?*

- ☐ 1 Non
- ☐ 2 Oui, moins de 2 heures par semaine
- ☐ 3 Oui, 2 heures et plus par semaine

➥ **Si oui** (quel que soit le nombre d'heures) :

- Combien de fois par semaine **en moyenne** ? ☐☐
- Depuis combien d'années ?
  - ☐ 1 Moins d'1 an
  - ☐ 2 1 an ou plus
    - ➥ Indiquez depuis combien d'années : ☐☐ année(s)

B/ Contraintes posturales

Les questions 42 à 47 se rapportent à une journée typique de travail.

42. *Comment évaluez-vous l'intensité des efforts physiques de votre* ***travail au cours d'une journée typique*** *de travail ?*

Pour cette question, présentez l'échelle ci-dessous au consultant.

Cochez le chiffre correspondant à votre choix sur l'échelle de 6 à 20 ci-dessous, qui va de « pas d'effort du tout » à « épuisant » :

- ☐ 6 pas d'effort du tout
- ☐ 7 extrêmement léger
- ☐ 8
- ☐ 9 très léger
- ☐ 10
- ☐ 11 léger
- ☐ 12
- ☐ 13 un peu dur
- ☐ 14
- ☐ 15 dur
- ☐ 16
- ☐ 17 très dur
- ☐ 18
- ☐ 19 extrêmement dur
- ☐ 20 épuisant

43. *Au cours d'une journée typique de travail*

	Jamais ou presque jamais	Rarement (moins de 2 heures par jour)	Souvent (2 à 4 heures par jour)	Toujours ou presque
Etes-vous debout ?	☐ 1	☐ 2	☐ 3	☐ 4
Devez-vous répéter les mêmes actions plus de 2 à 4 fois par minute ?	☐ 1	☐ 2	☐ 3	☐ 4
Pouvez-vous interrompre votre travail ou changer de tâche ou d'activité pendant 10 minutes ou plus chaque heure ?	☐ 1	☐ 2	☐ 3	☐ 4
Pouvez-vous quitter votre travail des yeux pendant quelques secondes en dehors des pauses ?	☐ 1	☐ 2	☐ 3	☐ 4
Devez-vous vous agenouiller ou vous accroupir ?	☐ 1	☐ 2	☐ 3	☐ 4
Devez-vous vous pencher en avant ou sur le côté régulièrement ou de manière prolongée ?	☐ 1	☐ 2	☐ 3	☐ 4
Devez-vous conduire un engin de chantier, un tracteur, un chariot automoteur ou autre machine mobile sur votre lieu de travail ? (hors véhicule, cf. point suivant)	☐ 1	☐ 2	☐ 3	☐ 4
Devez-vous conduire un véhicule (automobile, camion, autocar, autobus, ambulance, deux-roues motorisé…) sur la voie publique en excluant le trajet domicile-travail ?	☐ 1	☐ 2	☐ 3	☐ 4

Appendix III. Constances Cohort Study - Diet questionnaire

III. ALIMENTATION

7. *Pensez-vous que votre alimentation est équilibrée ?*

Tout à fait ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ Pas du tout
A B C D E F G H

8. *Pour vous, manger représente avant tout : (plusieurs réponses possibles)*

- ☐ 1 Une chose indispensable pour vivre
- ☐ 1 Un moyen de conserver la santé
- ☐ 1 Un plaisir gustatif
- ☐ 1 Un bon moment à partager avec d'autres
- ☐ 1 Une contrainte

9. ***Habituellement, en semaine,*** *où prenez-vous* ***le plus souvent*** *votre repas de* ***midi*** *?*
(Considérez les jours où vous travaillez si vous exercez un emploi ou les jours de cours si vous êtes scolarisé(e) ou étudiant(e))

- ☐ 1 Chez vous
- ☐ 2 À la cantine (restaurant d'entreprise, école, restaurant universitaire…)
- ☐ 3 Sur le lieu de travail (mais pas à la cantine)
- ☐ 4 Chez des amis
- ☐ 5 Au fast food, dans un snack
- ☐ 6 Au restaurant, pizzeria, cafétéria
- ☐ 7 Au café, bistrot
- ☐ 8 Dans la rue ou dans un parc
- ☐ 9 Autre

10. ***Actuellement,*** *suivez-vous un régime alimentaire ?*

☐ 1 Oui ☐ 2 Non

➥ **Si oui** :

- Ce régime vous a-t-il été prescrit par un(e) professionnel(le) de santé : médecin généraliste, médecin spécialiste (nutritionniste ou endocrinologue), diététicien(ne) ?

  ☐ 1 Oui ☐ 2 Non

- Pour quelle raison suivez-vous ce régime ? (plusieurs réponses possibles)

  - ☐ 1 Pour une raison médicale sans lien avec un problème de surpoids (allergie alimentaire, régime sans sel...)
  - ☐ 1 Pour maigrir
  - ☐ 1 Pour ne pas prendre de poids
  - ☐ 1 Pour rester en forme
  - ☐ 1 Autre(s) raison(s) (végétarien, conviction personnelle ou religieuse...)

11. ***Actuellement,*** *considérez-vous que vous êtes :*

- ☐ 1 Beaucoup trop maigre
- ☐ 2 Un peu trop maigre
- ☐ 3 D'un poids normal
- ☐ 4 Un peu trop gros(se)
- ☐ 5 Beaucoup trop gros(se)
- ☐ 6 Ne sait pas

12. ***Habituellement**, à quelle fréquence consommez-vous les aliments ou boissons suivants, quel que soit leur mode de conservation (**frais, en conserve ou surgelé**), le moment de consommation (repas ou hors repas) et le lieu (domicile ou hors domicile) ?*

ℹ Pour les questions marquées d'une *, répondez en excluant les produits allégés ou lights. Ils font l'objet de questions spécifiques.

• Aliments :	Jamais ou presque	Moins d'1 fois par semaine	Environ 1 fois par semaine	2 à 3 fois par semaine	4 à 6 fois par semaine	1 fois par jour ou plus Dans ce cas, combien de fois ou d'unités par jour ?
Viande (bœuf, veau, agneau, porc...)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Volaille (poulet, dinde...)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Poisson ou fruits de mer	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Œufs	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Charcuterie et abats (jambon, pâté, lard, boudin, andouillette...)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Lait	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Produits laitiers (petits suisses, yaourts, fromage blanc...) *	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Desserts sucrés (entremets, crèmes desserts, mousses, glaces...) *	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Produits laitiers et desserts **allégés (à 0 % ou 20 %)**	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Fromages *	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Fromages **allégés**	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Pain blanc, biscottes	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Pain **complet, intégral, au sarrasin, aux céréales, de seigle,** biscottes **complètes**	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Céréales pour le petit-déjeuner	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Féculents (pâtes, pommes de terre, riz, semoule...)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Riz **complet ou brun**, pâtes **complètes**...	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Légumes secs (lentilles, haricots blancs, pois chiches...)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Légumes crus (crudités) ou cuits	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Fruits frais (y compris fruits pressés)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Plats cuisinés du commerce (en conserve, surgelés, traiteur : couscous, cassoulet, choucroute...) *	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Plats cuisinés du commerce **allégés**	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Plats de restauration rapide (hamburgers, kebab, sandwich, pizza, quiches...)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

	Jamais ou presque	Moins d'1 fois par semaine	Environ 1 fois par semaine	2 à 3 fois par semaine	4 à 6 fois par semaine	1 fois par jour ou plus Dans ce cas, combien de fois ou d'unités par jour ?
Aliments frits (frites, chips, beignets, viandes ou poissons panés...)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Biscuits salés, cacahouètes, et autres produits apéritifs	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Pâtisseries, gâteaux, viennoiseries	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Biscuits sucrés, barres chocolatées ou de céréales, bonbons, chocolat... *	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Biscuits sucrés, barres chocolatées ou de céréales, bonbons, chocolat... **allégés**	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Beurre, margarine (au petit déjeuner, en accompagnement, dans la préparation des repas)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Huile (assaisonnemnent ou cuisson)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

• Boissons :	Jamais ou presque	Moins d'1 fois par semaine	Environ 1 fois par semaine	2 à 3 fois par semaine	4 à 6 fois par semaine	1 fois par jour ou plus Dans ce cas, combien de verres ou de tasses ?
Café	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Thé	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Jus ou nectar de fruits du commerce	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Soda (Orangina, Schweppes...), boisson aromatisée sucrée (Oasis, Ice tea...) *	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Soda, boisson aromatisée **light ou zéro**	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Boisson énergisante (Red bull, Monster...) (à l'exclusion du café et des boissons pour sportifs)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

13. *Combien de sucre (blanc, brun, roux...) consommez-vous* ***par jour*** *(café, thé, yaourt...) ? (nombre de morceaux ou de cuillerées à café)*

☐ 1 Jamais ou rarement ☐ 2 1 ou 2 ☐ 3 3 ou 4 ☐ 4 5 ou plus

14. *Combien de sucre allégé ou édulcorant (aspartame, stévia, sirop d'agave ...) consommez-vous* **par jour** *(café, thé, yaourt...) ? (nombre de morceaux, sucrettes ou de cuillerées à café)*

☐ 1 Jamais ou rarement ☐ 2 1 ou 2 ☐ 3 3 ou 4 ☐ 4 5 ou plus

15. *Aimez-vous manger très salé ou resalez-vous vos plats avant de les avoir goûtés ?*

☐ 1 Oui ☐ 2 Non

16. *Quel type de matière grasse utilisez-vous le plus souvent pour cuire les aliments ? (une seule réponse)*

☐ 1 Beurre ☐ 2 Beurre allégé ☐ 3 Huile ☐ 4 Margarine ☐ 5 Autre

➥ **Si vous utilisez de la margarine**, précisez quel type :

☐ 1 Margarine standard

☐ 2 Margarine enrichie en oméga 3 (Saint-Hubert Oméga 3, Planta Fin Oméga 3...)

☐ 3 Margarine enrichie en stérols végétaux (Fruit d'Or Pro-Activ', Saint-Hubert Cholégram...)

17. *Quels types d'huile utilisez-vous le plus souvent pour l'assaisonnement ou la cuisson ? (deux réponses maximum)*

☐ 1 Tournesol ☐ 3 Colza ☐ 5 Olive

☐ 2 Arachide ☐ 4 Huile de mélange (type Isio 4) ☐ 6 Autre

IX. Constances Cohort Study - Sleep questionnaire

IX. VOTRE SOMMEIL

56. *Au cours **du dernier mois**, indiquez combien de jours :*

	Jamais	1 à 3 jours	4 à 7 jours	8 à 14 jours	15 à 21 jours	22 à 31 jours
Vous avez eu des difficultés à vous endormir	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Vous vous êtes réveillé(e) plusieurs fois par nuit	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Vous vous êtes réveillé(e) beaucoup trop tôt sans pouvoir vous rendormir	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Vous vous êtes réveillé(e) après une nuit de sommeil de durée habituelle en vous sentant fatigué(e) ou épuisé(e)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

57. *Au cours de la **dernière semaine** (en dehors du week-end), combien de temps avez-vous dormi **en moyenne** par nuit ?*

- ☐ 1 Moins de 5 heures
- ☐ 2 5 heures
- ☐ 3 5 heures 30
- ☐ 4 6 heures
- ☐ 5 6 heures 30
- ☐ 6 7 heures
- ☐ 7 7 heures 30
- ☐ 8 8 heures
- ☐ 9 8 heures 30
- ☐ 10 9 heures
- ☐ 11 9 heures 30
- ☐ 12 10 heures et plus

XIII. Constances Cohort Study - Tobacco questionnaire

XIII. CONSOMMATION DE TABAC ET DE CIGARETTE ÉLECTRONIQUE

104. *Au cours de **votre vie,** avez-vous déjà consommé du tabac (au moins 100 cigarettes (soit 5 paquets) ou 50 cigarillos ou 50 pipes ou 25 cigares) ?*

☐ 1 Oui ☐ 2 Non

➥ **Si non,** passez **directement** à la **question 107, page 27**.

➥ **Si oui** :

- A quel âge avez-vous commencé ? ☐☐ ans
- Fumez-vous encore **actuellement** ? ☐ 1 Oui ☐ 2 Non
  - ➥ **Si non,** à quel âge avez-vous arrêté **(dernier arrêt)** ? ☐☐ ans

105. *Si vous vous êtes **déjà arrêté puis avez recommencé**, combien de temps **au total** ces périodes d'arrêt ont-elles duré ?*

☐ 1 Moins d'1 an

☐ 2 1 an ou plus

➥ Indiquez combien d'années **au total** : ☐☐ année(s)

106. ***Sur l'ensemble de vos périodes de consommation**, avez-vous fumé :*

- **La cigarette** (hors cigarette électronique) : ☐ 1 Oui ☐ 2 Non
  - ➥ **Si oui** :
    - Pendant combien d'années ?
      - ☐ 1 Moins d'1 an
      - ☐ 2 1 an ou plus
        - ➥ Indiquez combien d'années : ☐☐ année(s)
    - Combien de cigarettes par jour **en moyenne** ?
      - ☐ 1 Moins d'1 cigarette par jour
      - ☐ 2 1 cigarette ou plus par jour
        - ➥ Indiquez combien de cigarettes par jour **en moyenne** : ☐☐ cigarette(s)
- **Le cigarillo, le cigare, la pipe** : ☐ 1 Oui ☐ 2 Non
  - ➥ **Si oui** :
    - Pendant combien d'années ?
      - ☐ 1 Moins d'1 an
      - ☐ 2 1 an ou plus
        - ➥ Indiquez combien d'années : ☐☐ année(s)
    - Combien par jour **en moyenne** ?
      - ☐ 1 Moins d'1 par jour
      - ☐ 2 1 ou plus par jour
        - ➥ Indiquez combien par jour **en moyenne** : ☐☐

XV. Constances Cohort Study - Alcohol questionnaire

XV. CONSOMMATION DE BOISSONS ALCOOLISÉES

110. *Au cours de **votre vie**, avez-vous déjà consommé des boissons alcoolisées (vin, apéritif, cidre, bière…) ?*

☐ 1 Oui ☐ 2 Non

➥ **Si non,** passez **directement** à la **partie suivante :** XVI. VIE AU TRAVAIL, page 31

111. ***Habituellement,** à quelle fréquence consommez-vous des boissons alcoolisées ?*

☐ 1 1 à plusieurs fois par semaine

➥ Indiquez combien de jours par semaine : ____ jour(s)

☐ 2 2 à 3 fois par mois

☐ 3 1 fois par mois ou moins

☐ 4 Jamais

➥ Indiquez pourquoi :

☐ 1 Pour des raisons de santé

☐ 2 Par goût

☐ 3 Autre

ℹ Si vous ne consommez **jamais** de boissons alcoolisées, passez **directement** à la **question 119, page 30**.

Les 3 questions suivantes portent sur votre consommation de boissons alcoolisées standard.
Pour y répondre, référez-vous aux illustrations ci-dessous.

Une boisson alcoolisée standard

25 cl de bière à 5°	12,5 cl de champagne à 12°	12,5 cl de vin à 12°	2,5 cl de pastis à 45°	4 cl de digestif à 40°	4 cl de whisky à 40°	2,5 cl de digestif à 45°

Tous ces verres standards contiennent la même quantité d'alcool (10 grammes)
Une « chope » de bière (de 50 cl) ou une double « dose » d'alcool fort équivalent à deux boissons standard.

112. ***Les jours où vous consommez de l'alcool,** combien de boissons alcoolisées standard buvez-vous **en moyenne au cours d'une journée ?***

____ boisson(s) alcoolisée(s) standard

113. *A quelle fréquence vous arrive-t-il de boire **six boissons alcoolisées standard ou plus** au **cours d'une même occasion** ?*

☐ 1 Jamais
☐ 2 Moins d'une fois par mois
☐ 3 Chaque mois
☐ 4 Chaque semaine
☐ 5 Chaque jour ou presque

114. *Au cours des **12 derniers mois,** quel est le nombre **maximal** de boissons alcoolisées standard que vous avez consommées en **1 jour ?***

☐☐ boisson(s) alcoolisée(s) standard

115. *En pensant aux **12 derniers mois,** répondez aux questions suivantes :*

	Jamais	Moins d'une fois par mois	Chaque mois	Chaque semaine	Chaque jour ou presque
Combien de fois n'avez-vous pas pu faire ce que normalement vous auriez dû faire, parce que vous aviez bu ?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Combien de fois avez-vous observé que vous n'étiez plus capable de vous arrêter de boire après avoir commencé ?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Combien de fois avez-vous eu un sentiment de culpabilité ou de regret après avoir bu ?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Combien de fois avez-vous été incapable de vous souvenir de ce qui s'était passé la veille parce que vous aviez bu ?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Après une période de forte consommation, avez-vous dû boire de l'alcool dès le matin pour vous remettre en forme ?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

116. *Au cours des **12 derniers mois,** vous êtes-vous blessé ou avez-vous blessé quelqu'un parce que vous aviez bu ?*

☐ 1 Oui ☐ 2 Non

➥ **Si non,** cela vous est-il arrivé **auparavant** ? ☐ 1 Oui ☐ 2 Non

117. *Au cours des **12 derniers mois,** est-ce qu'un parent, un ami, un médecin ou un autre professionnel de la santé s'est déjà préoccupé de votre consommation d'alcool ou vous a conseillé de la diminuer ?*

☐ 1 Oui ☐ 2 Non

➥ **Si non,** cela vous est-il arrivé **auparavant** ? ☐ 1 Oui ☐ 2 Non

118. ***Pouvez-vous décrire votre consommation de boissons alcoolisées standard au cours de la dernière semaine ?***

ℹ Si vous **n'avez pas consommé de boisson alcoolisée les jours indiqués,** cochez « Aucune boisson alcoolisée ».

	Aucune boisson alcoolisée	Bière, cidre	Vin, Champagne	Alcool fort	Apéritif	Premix*	Cocktail
			Rouge, blanc, rosé	Whisky, Vodka, Pastis, etc.	Suze, Martini, etc.		Gin tonic, Punch, Téquila sunrise, etc.
		Nb de verres standard	Nb de verres standard	Nb de verres standard	Nb de verres standard	Nb de bouteilles 30 cl	Nb de verres standard
Du Lundi au Jeudi (Nombre de verres par jour en moyenne)	☐ 1						
Vendredi	☐ 1						
Samedi	☐ 1						
Dimanche	☐ 1						

*Premix : petite bouteille de 30 cl contenant un mélange d'alcool et de soda d'environ 5° : Smirnoff Ice™, Boomerang™, etc.

119. ***À quel âge avez-vous bu au moins une boisson alcoolisée standard pour la première fois ?*** ans

120. ***Avez-vous déjà été saoul(e) ?***

☐ 1 Oui ☐ 2 Non

➥ **Si oui,** quel âge aviez-vous **la première fois ?** ans