RHINESSA studien

Lungehelseundersøkelsens Generasjonsstudie

- oppfølgingsstudie okt 2020

Standard operating procedures for collection of environmental samples

Home environment (dust) sampling

Dust collection device (VacuuMark, A-B Miljø A/S, Bjerringbro, Denmark) with filter is attached on the nozzle of the vacuum cleaner. The pore-size of the filter is 5-6 µm (% of particles that is retained on the filter): 75% of particle size 0.3-0.5 µm; 81% of the particle size of 0.5-1.0 µm; 95% of particles of size 1-10 µm; and 100 % of particles above 10 µm.

Sample collection is accomplished by slowly and lightly drawing the dust collection device above the surface of rugs, upholstery, wood floors, windowsills, and furniture in the most frequently used rooms of the house, usually 4-5 rooms including hallways. A separate dust sample can be collected from the mattress of the participants bed.

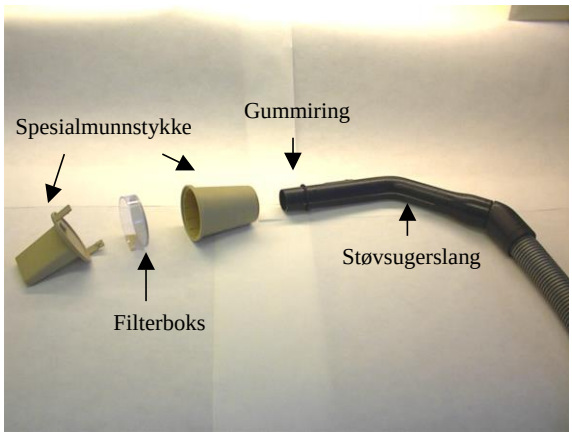
The participants are asked to fill in an information sheet with information on the type of samples that have been collected, including information regarding duration of sampling and what kind of furniture and floors were sampled (e.g. carpet floors, wooden floors or tiles). After sample collection, the participants ship the sampling device and samples back to the laboratory (Seksjon for allergi and proteinanalyser, Haukeland University Hospital). The filter boxes for the dust sample collection, are weighed before and after sampling. Once received in the lab, the samples will be weighed and stored at -4°C until they can be shipped on dry ice to the lab for analyses [2].

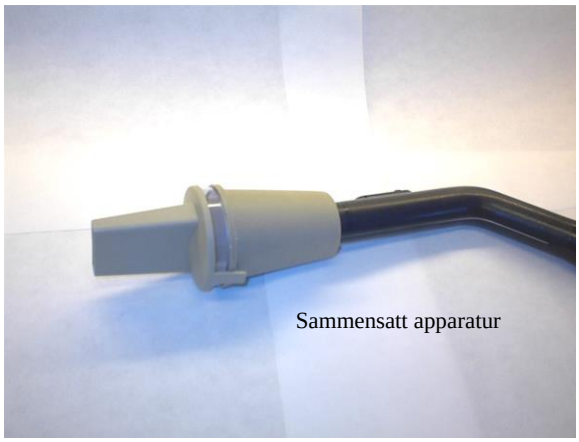
We are collecting two types of samples: Mattress dust and samples from the rooms mostly used by the occupants: vacuum-cleaning surfaces of the floors and from surfaces above the floors, such as shelves, furniture and windowsills. The addition of surfaces besides floors gives a higher probability that enough dust is sampled and that it is representative for the environment that the participants lives in. Using upholstered furniture gives a better picture of exposure from a longer period of time (than from floors which likely represents short time exposure). Furthermore, floors might have insufficient amount of dust, in particular Norwegian homes where most houses have wooden floors and not carpets (serve as reservoirs). Elevated surface dust contains finer particles which more readily absorb chemicals from the ambient environment [3].

The two next pages shows the information which follows the dust sampling devices

Veiledning for støvoppsamling

1. Hvis mulig, skift til ny støvsugerpose før prøvetaking (gjelder ikke sentralstøvsuger).
2. Montér vedlagte gummiring på støvsugerslangen.
3. Fest filterboksen (uten lokk) mellom de to delene av munnstykket (se Figur 1). Fest slangen til åpningen på spesialmunnstykket (se Figur 2).





Figur 1

Det følger 2 filterbokser med denne pakken. Du kan velge å ta en prøve fra madrassen i sengen og en samleprøve fra oppholdsrom.

Madrass: Ved støvoppsamling fra madrass, er det viktig å fjerne lakenet og deretter støvsuge madrassen og kantene godt. Ved bruk av middtrekk beholdes dette på. Støvsug hele madrassen i 4 minutter. Bruk 500 W effekt eller lavere på støvsugeren, dersom denne er innstillbar. Pass på at ikke motoren på støvsugeren går varm, ta i såfall en liten pause før du fortsetter. Oppgi madrassmål (bredde og lengde) i prøvetakingsskjema på neste side.

Oppholdsrom: Støvsug gulv, tepper og andre områder f.eks tekstil-/stoppede møbler som sofa og stoler i rommene som du oppholder deg mest i. Bruk 500 W effekt eller lavere på støvsugeren, dersom denne er innstillbar. Pass på at ikke motoren på støvsugeren går varm, ta i såfall en liten pause før du fortsetter.

4. Etter støvsuging er utført, demonteres spesialmunnstykket og filterboksen. Husk å holde spesialmunnstykket rettet oppover ved demontering slik at ikke støvet faller ut. Sett lokk på filterskålen og legg boksen i vedlagte lynlåspose. Dersom du har mer enn en prøve, merk posen med oppsamlingssted. Filter boks, spesialmunnstykket, gummiring, og utfylt prøvetakingsskjema (side 2), returneres til Haukeland Universitetssykehus i den ferdig adresserte konvolutten. Du trenger ikke betale porto, den betales av oss ved mottak av returkonvolutten.

Støvprøve

Ta gjerne kontakt dersom du lurer på noe i forbindelse med prøvetakingen
Prosjektleder: Randi J. Bertelsen; E-mail: randi.j.bertelsen@uib.no, Mobil: 99674046

Informasjon om støvsugeren som ble benyttet (merke, modell, effekt benyttet, sentralstøvsuger eller vanlig støvsuger):

Hvor mange personer bor i boligen nå: ……

Informasjon om prøven/prøvene som ble samlet inn (fyll inn så godt som mulig om rom/møblene prøven er tatt fra):

Prøve nr	Rom (soverom, stue, annet)	Møbel (seng, teppe, sofa, tregulv etc)	Dersom seng: Oppgi bredde og lengde i cm	Madrassen har midd-trekk	Alder på møbelet eller madrassen	Dersom kjent, oppgi: Tekstiltype for teppe/sofa Type madrass

Andre kommentarer/bemerkning om støvprøvetaking:

Analytical procedures for environmental (dust) samples

The dust sample will be weighed and then split into aliquots for extraction and analyses by two analytical methods: one for chemical analyses and one for bacterial sequencing.

Chemical analyses

For the chemical analyses the dust will be extracted by solid phase extraction (SPE) [4]. The analytes will first be extracted from the sample matrix by methanol, and then SPE procedure is applied as a second step.

Microbiome analyses

For the bacterial DNA analyses, the dust will be extracted with sterile buffer with 0.05% Tween 20. This extraction procedure can also be used for allergen quantification. For bacterial quantification will use qPCR for bacterial DNA yield, 16S rRNA Illumina HiSeq sequencing and reduced metagenome sequencing (RMS) for bacterial determination. Similar analyses will be performed on the saliva and skin microbiome samples (see below).

The reduced metagenome sequences can be used to identify **antibiotic resistance genes (ARGs)**. The ARGs can be identified through databases (e.g. ResFinder or AMR tools). The RMS was successfully applied on gut samples and used with ResFinder [5] assignment to identify antibiotic resistance genes [6].

Standard operating procedures for collection of urine

Urine will be collected on site during clinical examination or from samples collected at home.

Samples collected during clinical examination will be frozen directly without buffer or preservatives in 5 ml polypropylene tubes (Falcon tubes).

The urine samples collected at home will be collected and stored in Norgen Biotek Urine Collection and Preservation Tubes (Product # 18118, Norgen Biotek Corporation, Thorold, ON, Canada). The tubes contain Norgen's Urine Preservative in dried format. The user simply collects urine into the tubes and mixes gently until the orange preservative pellet in the tube has dissolved. The urine preservative prevents the growth of Gram-negative and Gram-positive bacteria and fungi, and also inactivates viruses allowing the resulting non-infectious samples to be handled and shipped safely. The components of the Urine Preservative allow samples to be stored for over 2 years at room temperature with no detected degradation of urine DNA, RNA or proteins.

Urine collection tubes and preservatives have in some cases been found to contain chemicals that might interfere with quantification of phenolic compounds (the antibacterial chemicals) in urine, as was found for the MoBa study, and in particular relevant for bisphenol A [7]. However, the preservative used in urine collection tubes (Norgen Biotek) contain EDTA and Sodium azide (Na-N=N=N) and no phenolic compounds and thus should not interfere with the phenolic quantification. Also, the plastic that are used (Falcon tubes) is polypropylene plastic (BPA free). To further control for this, we will store urine from the same participant (participants that attend the clinical examination at the hospital) in the two types of tubes: without any preservative and in the tube with preservative. This will be used as control samples for background contamination. In addition to control (duplicate) stored samples, we also store field blank samples: distilled water is added to the tubes with the same procedure and material as is used for the regular urine sampling procedures. The field blanks are analyzed together with the urine samples to account for background contamination. Before the urine is frozen (on site collection) and immediately upon receiving the urine collection tubes from home collection, urine specific gravity will be measured in urine. The urine specific gravity can be used to account for urine dilution.

Analytical procedures of urine

Environmental phenols and parabens will be quantified in urine by on-line solid-phase extraction-high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) or Automated on-line column-switching HPLC-MS/MS [8]. The urine samples will be analyzed at Department of environmental exposure and epidemiology, Norwegian Institute of Public Health.

The two next pages shows the information which follows urin sampling device as well as the report to be following the sample in the return envelope

Prosedyre for innsamling av urin

For å unngå at bakterier fra hudfolder i kjønnsorganet forurenser prøven, skal urinprøven tas som midtstrømsprøve.

Første morgenurin er ønskelig.

Midtstrømsprøve

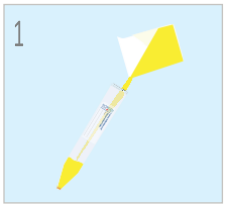
- Kvinner holder kjønnsleppene fra hverandre
- Menn trekker hudfolden tilbake

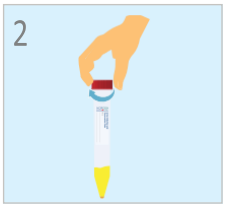
Urinbeger
100 ml

Prøveglass
med pellet
15 ml

1. La første del av urinstrålen gå i toalettet
2. Samle deretter urin i det sterile urinbegeret som medfølger.
3. Hell urin (ca 15 ml) over i prøveglasset. La resten av urinen gå i toalettet.
4. Når urinen er i røret, skru korken godt til
5. Vend røret forsiktig flere ganger til den oransje pelleten i bunnen av røret er oppløst
6. Oppbevar urinen i romtemperatur til du er klar til å returnere prøvepakken

I skjema som medfølger prøvepakken skal det noteres dato og klokkeslett for når urinprøven ble tatt, samt eventuelle andre merknader du har til prøvetakingen.





Prøvetakingsskjema for innsamling av urin

Urin-prøve

Første morgenurin er ønskelig

Dato for innsamling (e.g. 26/04/2020): _____ / _____ /_____

Tidspunkt for innsamling (e.g. 07:15): _____: _____

Var dette første morgenurin?

NEI ☐

JA ☐

Andre kommentarer:

_____

Standard operating procedures for collection of saliva

Saliva will be collected on site during clinical examination or from samples collected at home.

Samples collected during clinical examination will be frozen directly without sterile PBS in 5 ml sterile polypropylene tubes (Falcon tubes).

The saliva samples collected at home will be collected and stored in Norgen Saliva DNA Collection and Preservation Devices (Product # RU49000, Norgen Biotek Corporation, Thorold, ON, Canada). The saliva DNA in preserved samples is stable for more than 2 years at room temperature. The buffer prevents the growth of Gram-negative and Gram-positive bacteria and fungi, and also inactivates viruses allowing the resulting non-infectious samples to be handled and shipped safely
https://norgenbiotek.com/product/saliva-dna-collection-and-preservation-devices-50

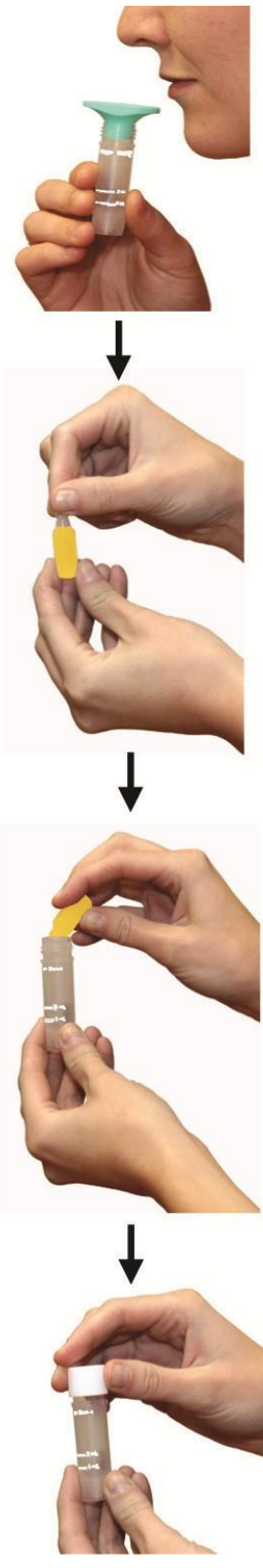
Analytical procedures of saliva

Microbiome analyses

Quantitative PCR will be applied for bacterial DNA yield in the saliva samples. In addition, 16S rRNA Illumina HiSeq sequencing and reduced metagenome sequencing (RMS) will be applied for bacterial determination (similar to procedures described for dust samples).

The two next pages shows the information which follows saliva sampling device as well as the report to be following the sample in the return envelope

Prosedyre for innsamling av spytt



Det er ønskelig at spytt samles inn om morgenen – før tannpuss. Ikke spis, tygg tyggis, drikk (kun skyll munnen med vann som beskrevet under), røyk eller bruk snus 30 minutter før spyttoppsamling. Unngå å berøre innvendig på oppsamlingstrakten, prøvetakingsrøret og korken.

1. Skyll munnen med vann og vent 10 minutter
2. Bøy hodet ned og la spyttet renne ned i oppsamlingstrakten (lys grønn trakt på bilde 1) fra nedre leppe. Fortsett til mengden flytende spytt (uten bobler) i røret når 2 ml linjen.
3. Vri og fjern spissen på konserveningsampullen (rør med gul væske)
4. Klem konserveringsampullen for å få alt innholdet med i oppsamlingsrøret.
5. Skru lokket på oppsamlingsrøret.
6. Rist røret godt i 10 sekunder for å blande spytt og konserveringsmiddel
7. Konserveringsampullen kan kastes i vanlig restavfall, mens plastemballasjen kan resirkuleres i plastavfall.
8. Oppbevar spyttprøven i romtemperatur til du er klar til å returnere prøvepakken

I prøvetakingsskjema som medfølger prøvepakken skal det noteres dato og klokkeslett for når spyttprøven ble tatt.

Prøvetakingsskjema for innsamling av spytt

Prøvetakingsskjema fylles ut når prøvene tas og returneres i returkonvolutt sammen med prøvene

Spytt-prøve

Det er ønskelig at spytt samles inn om morgenen

Dato for innsamling (e.g. 26/04/2020): _____ / _____ /_____

Tidspunkt for innsamling (e.g. 07:15): _____: _____

Hadde du spist, drukket noe, røykt eller brukt snus før innsamling av spytt?

NEI ☐

JA ☐

Hvis ja, hva og hvor lenge før spyttinnsamling:

_____

_____

Semen sampling

Semen will be collected at home. The samples will be collected in sterile polypropylene tubes (Falcon tubes 50ml https://nordicbiosite.com/product/325-2115-150J/50-ml-Centrifuge-Tube) and then poured into smaller sterile polypropylene tubes (15ml https://www.sarstedt.com/en/products/diagnostic/urine/tubes/product/62.554.502/) in which 5 ml of a preservative of DNA/RNA shield solution has been added in advance https://www.nordicbiosite.com/product/BioSite-R1100-1L-PUR/DNARNA-Shield-1000-ml. The preservative will keep RNA stable for 30 days and DNA stable for up to two years at room temperature. When the samples arrive at the lab they will be frozen at– 80 °C.

Analytical procedures of semen/seminal fluid

Epigenomics analyses

DNA methylation will be assessed with the Illumina CytoSNP-850K BeadChip arrays (Illumina, Inc., CA, USA), which interrogates the methylation status of 853,307 CpG sites using a standard protocol. Quality control, subsequent filtering, summarization and normalization of raw-methylation data will be performed using established pipelines.

Transcriptomics analyses

Expression data will be generated on Illumina HiSeq platform. The generated reads will be analysed through established bioinformatics and statistical pipelines, using various tools to read and map the sequences (Bowtie2, version 2. 2.513 and TopHat, version 2.0.1314); and further to guide transcript assembly and to obtain gene expression level (Cufflinks, version 2.2.1 (ref 3)).

The two next pages show the information provided to the participants on procedure as well as the report to be returned with the sample.

Prosedyre for innsamling av sæd

Sædprøven tas helst 2 dager etter siste ejakulasjon/samleie.

7. Vask hender og penis og skyll godt.
8. Sædprøven tas ved masturbasjon. Kondom, glidemiddel eller andre produkter må ikke brukes. Hele sædprøven tas i vedlagt stort glass.
9. Hell prøven så oppi det mindre prøveglasset med væske.
10. Skru korken godt til.
11. Vend prøveglasset forsiktig flere ganger.
12. Plasser prøveglasset i beskyttelsesglasset.
13. Oppbevar prøven i romtemperatur til du er klar til å returnere prøvepakken

I skjema som medfølger prøvepakken skal det noteres dato og klokkeslett for når sædprøven ble tatt.

➔ Stort glass
Beskyttelsesglass

→ Prøveglass med væske

→

Prøvetakingsskjema for innsamling av sæd

Prøvetakingsskjema fylles ut når prøvene tas og returneres i returkonvolutt sammen med prøvene.

Sæd-prøve

Det er ønskelig at sæd-prøve samles inn 2 dager etter siste ejakulasjon/samleie.

Dato for innsamling (f.eks. 26/06/2020): _____ / _____ /_____

Tidspunkt for innsamling (f.eks. 07:15): _____: _____

Kommentar til prøvetaking (f.eks. utilsiktet hendelse):

_____

_____

Skin swab sampling

The samples will be collected on site during clinical examination or at home.

The procedure for collection at the clinic is as follows: One sample will be taken from the flexion crease and another sample midway between the wrist and MCP-joints, using Catch-All™ Sample Collection Swabs moistened with sterile specimen collection fluid. The swab head will be inserted into the sterile tube containing approx. 300 µL of sterile BPS – or enough PBS to cover the collection head, and the tube will be frozen directly.

Samples collected from home with be collected and stored in Norgen Swab Collcetion and DNA Preservation System (Cat. 45690) https://norgenbiotek.com/product/swab-collection-and-dna-preservation-system. One sample will be taken from the flexion crease and another sample midway between the wrist and MCP-joints. The Swab Preservative prevents the growth of Gram-negative and Gram-positive bacteria and fungi, and also inactivates viruses allowing the resulting non-infectious samples to be handled and shipped safely. In addition, the Swab Preservative eliminates the need to immediately process or freeze samples and allows the samples to be shipped to centralized testing facilities at ambient temperatures.

Analytical procedures of skin swab samples

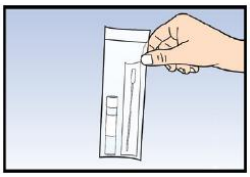
Microbiome analyses

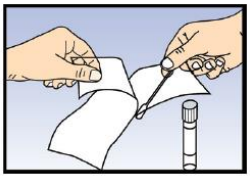
Quantitative PCR will be applied for bacterial DNA yield for the skin microbiome samples. In addition, 16S rRNA Illumina HiSeq sequencing and reduced metagenome sequencing (RMS) will be applied for bacterial determination (similar to procedures described for dust samples and saliva samples).

The two next pages shows the information which follows skin swab sampling device as well as the report to be following the sample in the return envelope

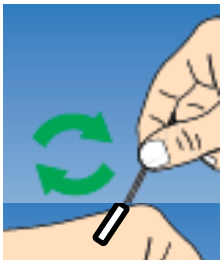
Prosedyre for innsamling av hudprøve

Det er ønskelig at prøven tas om morgenen før du har vasket deg, dusjet eller tatt på krem, bodylotion eller lignende på huden.

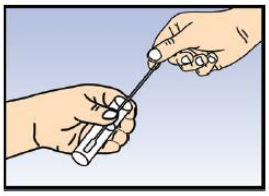




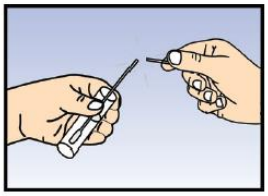
1. Åpne posen og ta ut den innpakkede vattpinnen og røret med konserveringsmiddel.
2. Åpne posen med vattpinne. **Det er viktig at du ikke berører tuppen av vattpinnen og ellers har så steril håndtering som mulig ved prøvetaking.**



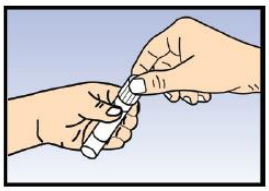
3. Plaser tuppen av vattpinnen på overflaten av håndbaken, roter pinnen og beveg den frem og tilbake i et halvt minutt for å samle så mye materiale som mulig.



4. Åpne forsiktig røret med konserveringsmiddel og før inn vattpinnen. Sjekk at tuppen av vattpinnen er i kontakt med væsken i røret.



5. Knekk pinnen på punktet som er avmerket på pinnen slik at vatthodet blir værende inne i røret.



6. Sett korken tilbake på røret og skru godt igjen. Vend på røret flere ganger for å bland vattpinnen med væsken i røret.

Oppbevar røret i romtemperatur til du er klar til å returnere prøvepakken.

I prøvetakingsskjema som medfølger prøvepakken skal det noteres dato og klokkeslett for når hudprøven ble tatt, og eventuelt andre bemerkninger til prøvetakingen.

Prøvetakingsskjema for innsamling av hudprøve

Prøvetakingsskjema fylles ut når prøvene tas og returneres i returkonvolutt sammen med prøvene

Hud-prøve

Det er ønskelig at prøven samles inn om morgenen

Dato for innsamling (e.g. 26/04/2020): _____ / _____ /_____

Tidspunkt for innsamling (e.g. 07:15): _____: _____

Har du hatt noen form for krem eller salve på huden?

NEI ☐

JA ☐

Hvis ja, hvilken og hvor lenge før prøvetaking:

_____

Andre bemerkninger til prøvetaking?

_____

Standard operating procedures for assessment of transepidermal water loss

Measurement of transepidermal water loss (TEWL) and stratum corneum (SC) hydration is important for assessing epidermal functions. TEWL is used as a research tool to objectively assess skin barrier function. TEWL as a measure of skin water barrier status has been validated in humans (Ye el al, Validation of GPSkin Barrier for assessing epidermal permeability barrier function and stratum corneum hydration in humans. Skin Res Technol. 2019; 25:25-29). As a research tool, TEWL enables objective and noninvasive measurement of one aspect of skin barrier function in dermatological research. TEWL elevation is a hallmark of atopic dermatitis (AD) and may precede clinical manifestation of the disease, suggesting that TEWL measures may be useful in guiding AD prevention strategies.

For each participant we will have two repeated measures on each of the following sites: one on the forearm (illustrated in the picture above), one on the volar side of the upper arm, and one on the back of the hand. Each measurement takes less than one minute, and the results are logged directly in the computer.

Instruction for fieldworkers for TEWL measurements

- Individuals should **acclimatize for 15–30 min before TEWL measurements**, with the skin at the measuring site left uncovered. The test person **should not eat or consume caffeine-containing drinks just before and during the measurements**.
- Measurement of **skin temperature** of the test person on the test site is recommended.
- Only TEWL values from the **same (or close-by) anatomical area** are expected to be comparable.
- **Relative humidity and temperature of the measuring room** should be recorded during TEWL measurements and stated in the report.
- If a climate room facility is available, the ambient room temperature should be regulated to **20–22 °C and the relative humidity lower than 60%.**
- TEWL should **not be measured under direct light sources**.
- The **measuring surface should be placed horizontally and the probe applied parallel to this surface**.
- The **contact pressure of the probe** on the skin should be kept **low and constant**.
- The measuring probe itself **should not be touched before and during measurements and can be handled with the electrical wire or a coating or by wearing gloves**.
- **TEWL values should be registered 30–45 s after application of the probe to the skin, preferably using a computer. With newer models, the time to reach a steady state is considerably shorter.**
- TEWL measurements should be **as short as possible in order to avoid occlusion**. Before each measurement, the zero value should be displaced.
- The use of the **protection covers** should be clearly stated in the protocol and publication.

Lung function Testing

Aim: to get at least one high quality spirometry measure for each participant. Spirometry testing offers important data on respiratory lung volume and function.

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

During a spirometry maneuver there is a small risk that the participant may faint and hurt him/herself while falling. **Participants must therefore perform the maneuver in 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 manouver.
Forced Expiratory Volume at One Second (FEV_1)	The amount of air that a person exhales during the first second of a forced expiratory manouver.
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 manouver.
The ratio of FEV_1 to the FEV_6 (FEV_1/FEV_6)	An alternative to the FEV_1/FVC ratio.

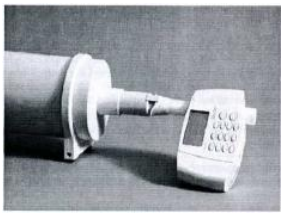
Location

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.

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 are 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 center **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 antimuscarin	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 maneuver, 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, center 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 (after week 23)

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 manouver.

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.

Baseline spirometry:

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 maneuver 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 dramatic blast out as fast as possible.

Number of blows to be conducted:

If after 5 attempts a grade A **or** grade B has been 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

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

Centers 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 coordinating center.

Data should be transferred to a local PC daily. From here they will be transferred to the coordinating center.

Quality Control Checks

At various points during the study the coordinating centers will request spirometry data from each center so that the Spirometry Curves arising from the testing each technician has done can be reviewed. Explicit instruction will be provided to each center at the time for the transfer of anonymous data and a brief report will be provided to each center.

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

Venesection

Aim: to collect blood samples for various testing of biomarkers for inflammation, antibiodies, metagenomics, epigenetics and CyTOF analyses. The blood samples will partly go into a biobank for long-term storage and future analyses. Certain analyses will be performed within a short timeframe.

The aim is to collect blood for the following samples (in order of priority) using standard venesection techniques. Staff should be trained and insured to carry out Venepuncture according to local requirements.

Vacutainer	Size	Processing	RPM/min	Bio-materials
EDTA	4 ml	Direct freeze		Wholeblood
Paxgene	2,5 ml	Invert tube 8-10 times Sore in room temperature for a minimum of 2 hours and max 72 hours before refrigeration or freezing		Wholeblood – freeze or refrigerate in the Paxgene tubes
Sodium heparine tubes	4 ml	Mix with Cytodelics whole blood processing kit.		Wholeblood (freeze in 2x270 µl cyotubes for CytoF) and 2x 1 ml tubes
Sodium heparine tubes	8,5 ml	Mix with Cytodelics whole blood processing kit and stimulate cells with for inhibition of cytokine secretion from the cells		1 ml whole blood in each of 15 ml tubes with stimulation and unstimulating buffer for each of the 2 stimulants*
EDTA	10 ml	Direct spin	4200 10 min	Plasma (freeze in 10 ml vacutainer)
EDTA	4 ml	Direct spin	4200 10 min	Plasma (2x 1 ml++, store in 2 ml tubes)
Gel-glas	8.5 ml	>30min <1 hour	3400 10 min	Sera (1 ml++, store in 2 ml tubes)

* 1) General stimulant PMA/ionomycin + BrefeldinA and 2) LPS + BrefeldinA (for inhibition of cytokine secretion from the cells);

Wholeblood stored in the Paxgene tubes will be used for transcriptomic analyses to identify the blood biomarkers associated with the phenotype of interest in the study, e.g., Covid infection or allergies. We can further associate the blood biomarkers with the immunological signatures in the germline, in the male participants from whom we have also collected sperm. We will also use these samples for epigenetics analyses for assessment with exposure to infection or chemicals. Further, we can assess how such epigenetic alterations can affect the gene expression (i.e., transcriptomics).

The plasma samples will be used to sequence miRNA. This main goal of this part of the project is to study the differences in the miRNAs, affecting the regulation of gene expression in individuals previously exposed to either the infection or chemicals.

The samples with wholeblood with Cytodelics whole blood processing kits and stimulation kits will be used to determine detailed cell type phenotyping by including cell surface markers and activation markers, and functional markers like intracellular cytokines for those cells that are stimulated before storage.

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

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.

Wash your hands and apply gloves.

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.

Preparation of serum sample

Equipment
Fridge
-20ºC freezer (with thermometer)
Swing head or fixed angle centrifuge
2ml (Sarstedt) storage tubes – (or tubes suitable for -20ºC 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

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.

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.

It is important to maintain an **impeccable sample logbook**. Copies of it will be required during sample shipment.