European Community Respiratory Health Survey III
(ECRHS III)

Clinical Protocol

Manual of Procedures for a multi-centre international study
to follow-up a cohort first recruited in 1992-1994 and, where applicable,
to interview for the first time a new sample of participants

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This is a common protocol for all centres – minor local variations may be introduced to comply with local research governance requirements.

Please note that in the UK the 'European Community Respiratory Health Survey is referred to as 'The East Anglian Respiratory Health Survey' in correspondence with participants

Overview

European Community Respiratory Health Survey I

In 1992/4 a population based random sample of 5000 adults aged 20-44 were identified from a suitable population based sampling frame (in the UK this was the Family Health Services Authority register of all those registered with local GPs in the participating areas). Each participant was sent a postal questionnaire asking nine questions on respiratory symptoms. A random sample of responders were then invited to a local testing centre to undergo further more detailed questionnaires, lung function tests including bronchial reactivity tests and skin prick tests. In addition a sample of those with symptoms strongly suggestive of asthma, but who had not been selected as part of the random sub-sample were invited to undergo similar assessments.

European Community Respiratory Health Survey II

Ten years later those participants who had taken part in the clinical assessment were recontacted and having completed a short postal questionnaire wee again invited to local testing centres to undergo further questionnaires and assessments including lung function tests, including bronchial reactivity testing.

European Community Respiratory Health Survey III

This protocol relates to the third wave of data collection on the cohort. All who underwent examination in ECRHS I have been recontacted by postal survey, and are now to be invited for a range of further tests to specifically assess the following:

The specific aims of the ECRHS III related to this application are to

1) Describe change in respiratory symptom prevalence in adults as they age
2) Assess change in IgE sensitisation to common allergens in adults as they age
3) Determine whether the prognosis of asthma is influenced by any observed change in atopic status
4) Assess whether atopic status and asthma as measured over a twenty year period is associated with lung function decline or the development of COPD in older adults
5) Describe the association of obesity and physical exercise with asthma, lung function, lung function decline and the prognosis of asthma

Throughout we will assess whether observed effects are similar in men and women and assess whether menopausal status influences associations within women.

This application will focus on these aims but data generated through this project will also be analysed to provide observational evidence of associations of lifestyle and environmental factors (eg occupation and air pollution) with atopy, respiratory symptoms, lung function decline and the development of COPD.

Enrichment with new participants

In some centres including the UK, a cross-sectional survey has been conducted on a sample of adults who are the same age as cohort members and who have been identified at random from current population based sampling frames. A random sample of 200 of these individuals and a sample of 200 with symptoms suggestive of asthma will also be invited to take part in the assessments that the cohort will undergo.

This repeat cross-sectional survey will increase the number of adults and the number of asthmatics for follow-up, in the event of ECRHS IV.

1. Participants and invitation to the clinic for testing

Any participant who has completed the ECRHS III short questionnaire is eligible for the ECRHS III clinical phase.

All participants should be provided with an appointment time and instructions on how to get to the clinic plus any additional material required by local research governance procedures.

Before attending the clinic all participants should be asked to

1) refrain from smoking for at least one hour
2) avoid eating a heavy meal for one hour before
3) avoid vigorous exercise for at least one hour before
4) refrain from taking their asthma medications before the visit if they have no symptoms (If ethical permission is granted to do this)

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

Please note that some participants may be taking medication with a long duration of action (24 hours) – if you are able to get ethical permission to tell them to cease taking these medications then please do so. However if it is not possible – please ensure you record recent medication usage correctly in the data sheets that are completed prior to lung function testing.

5) to wear clothing to the clinic that will make it easy for the tests to be performed with minimal disruption. For example to wear
    a. wear light clothing, avoiding tight collars and tight belts
    b. wear blouses and shirts where the sleeve can be rolled up for blood testing and skin prick tests
    c. wear sandals, if possible, and if not possible, to wear shoes that are easy to take off
    d. avoid wearing tights, and if wearing socks ensure these are easy to get on and off
    e. avoid wearing a lot of heavy metal jewellery

2.Overview of the protocol

Participants will be invited to the local testing centre for the following investigations which are listed below in the proposed order.

Explanation of procedure and consent
Main questionnaire
Getting ready for FENO, bioimpedence, spirometry, reversibility, questionnaire
FeNO
Height
Weight
Waist hip
Bioimpedence *(it may be appropriate to perform venesection at this moment)*
Skin prick testing
Lung function testing
Reading of skin prick tests
Completion of SF-36, AQLQ, ACT, body shapes, women's questionnaire while waiting for salbutamol effect
Food frequency questionnaire check
Exposure to sunlight questionnaire
It may be appropriate to perform venesection at this moment
Post broncholdilator measure of lung function

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

1. The following questionnaires MUST be self completed
   Food frequency questionnaire
   Exposure to sunlight questionnaire
   Body shapes
   Womens questionnaire
   SF-36
   AQLQ
   ACT

2. The SF-36, AQLQ and ACT MUST be completed AFTER the main questionnaire

3. Skin prick tests must be performed AFTER the main questionnaire and AFTER the FENO

4. Skin prick tests MUST be read at 15 minutes

5. Post bronchodilation FEV1 MUST be read AT LEAST 15 minutes after administration of bronchodilator

6. Where local permission is given the food frequency questionnaire 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.

3. Ethical permissions, getting consent and confidentiality

Centres to get ethical permission

All centres must comply with the local regulations regarding research and obtain all necessary permissions to conduct the research described in this document.

Specifically permission must be sought

For the clinical assessment itself

1) Collect questionnaire based information
2) Conduct the basic clinical procedures described herein including skin prick testing, reversibility testing and venesection

For the storage of biological samples

1) indefinite long term storage of **serum** for the testing of serum specific IgE to allergens in a centralised laboratory **in Amsterdam or any other centre** agreed by the Steering Committee.
2) indefinite long term storage of **serum** for the testing of other biological parameters in a centralised laboratory **in London or any other centre** agreed by the Steering Committee and following further ethical review as appropriate.
3) indefinite long term storage of **whole blood** for later extraction of DNA in the future. These tests may be conducted **in London or any other centre** agreed by the Steering Committee.
4) indefinite long term storage of a spot urine sample for measurement of pthalates, products of oxidative stress, metabonomics and any other tests relevent for cardiorespiraroty disease in the future. These tests may be conducted **in London or any other centre** agreed by the Steering Committee.

For the transfer of pseudo-annonymised data within the consortium

1) Transfer pseudo-annonnymised data to the coordinating centre in London
2) For the coordinating centre to transfer data to other members of the ECRHS as agreed by the steering committee of the study

Long term follow-up using available other databases (where applicable)

1) To electronically track the vital status of individuals using available mortality registers
2) To electronically track the health service utilisation of individuals using electronic health records
3) To search for the address of the individual in future years in order that they can be surveyed again

Data linkage with exposure information from geocodes

1) To geocode the residential history of the individual and from this derive exposure to a range of environmental pollutants.
2) To transfer pseudo-annonymised geocodes of participants to other centres to link with environmental exposure databases. These data will be transferred WITHOUT any health information and in a form that cannot be linked with any health information.

There will be local variation in the extent of permission provided at the beginning of the study and the topics which will need to be resubmitted to committees for approval when further details are available. However all centres must ensure that participants have completed a consent form that has a consideration of the above activities.

A model consent form is provided in this document. It is recognised that some centres may need to make changes.

All centres will send the coordinating centre a copy of the permission from their local ethical committee prior to commencing the clinical survey.

Informed consent from participants

All study participants must give their **written informed consent** for all aspects of the clinical visit or home visit. Study participants who are unable or unwilling to give written informed consent are ineligible to participate in the study.

Participants should be sent written information about what the study involves so that they can decide properly if they wish to take part. The content of this will vary with local regulation and practice but ideally it should:

- Have a clear and unambiguous heading
- Give the duration of the study
- Use language that is easy to understand
- Explain that the study is a research study, taking place in many European countries
- Explain what will happen if the participant takes part
- State that the visit will involve giving a blood sample and that the sample will be considered a donation/gift from participant.
- Explain that blood samples (separated from the participant's personal details) will be stored for later analysis
- Explain that genetic material in the blood sample may be analysed in the future, but only for research into asthma, allergic disorders, cardio-respiratory disease and nasal disorders asthma and allergy and for no other purpose
- State that all information shared with study staff will be kept confidential and that personal details will be separated from the collected data
- Explain that the collected data will be stored in encrypted format
- Provide information on any possible risks / benefits of taking part must be listed
- Explain what information the participant may receive after the visit
- Provide information on what will happen if something goes wrong at the visit. Any compensation arrangements should be detailed
- Explain what will happen to the results of the study
- State who is funding the research
- State who has given ethical approval for the study
- Give a contact for further information

A copy of the participant information sheet translated into English should be sent to the coordinating centre before the study begins in each centre.

Written consent will be obtained at the visit, before measurements and interview starts. The consent form should

- refer to the correct version number and date of the Participant Information Sheet that the participant has been given.
- reiterate that the study blood sample is given as a donation
- should clearly state that the participant gives their permission that his or her blood sample and **the genetic material in the sample** may be analysed in the future, on a number of occasions, as part of research in asthma, allergic disorders, cardio-respiratory disease and nasal disorders asthma and allergy (and not used for any other purpose).

Confidentiality

Participants' data must be kept confidential.

- Each study participant has a unique ID number and this should be used at all times.
- Study ID numbers that relate to any personal data (names and addresses) must be kept separated from any ID numbers attached to data collected during the clinical visit.
- Computers should be password protected and data should be stored in encrypted format

- No personal data (names and addresses) should be transferred out of centres. Transferred data should be identified by unique subject ID and centre ID **only.**
- Transfer of data between centres and the co-ordinating centre will be made electronically by secure file transfer
- Centres should keep all study forms, hard copies of data collected during the clinical visit data and other confidential information in secure locked areas. While in use, such forms should be kept private and safe as a priority
- Study participants may not be identified by name or any other means in any report, publication or presentation

Sample Consent form

Subject ID: Centre No:

Project – European Community Respiratory Health Survey III

Please initial each box you agree to. Put a line through any box you do not agree to.

Chief Investigator: xxxxxxxx

1	I confirm that I have read and understand the participant information sheet (Version 1.0 dated 1st May 2011) for the above study and have had the opportunity to ask questions.	
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or my legal rights being affected	
3	I understand that some **serum** and some **urine** will be stored anonymously and indefinitely for future analyses and agree to its use for research into **asthma, allergic disorders, cardio-respiratory disease and nasal disorders**.	
4	I understand that some **serum** and some **urine** will be stored anonymously and indefinitely for future analyses and I agree to its use for any health related purpose.	
5	I agree that the study researchers may store my DNA, the genetic material from my blood cells and may fully sequence it to examine the heritable part of **asthma, allergic disorders, cardio-respiratory disease and nasal disorders.**	
6	I agree that the study researchers may store my DNA and analyse it again at a later date **for any health related purpose**.	
7	I am happy for any residual blood to be used in any future research into **asthma, allergic disorders, cardio-respiratory disease and nasal disorders.**	
8	I am happy for any residual blood to be used in any future **health related research.**	
9	It is possible that researchers may wish to contact me again, to see if my health status has altered. I agree for health researchers to contact me again.	
10	I agree that researchers who wish to contact me may request permission to have my new address (if I have moved) from my general practitioner	
11	I give permission for researchers to be told the cause and date of my death (should I die) by the National Health Service Information Centre	
12	I agree to take part in the above study.	

_____ _____ _____

Name of Patient Date Signature

_____ _____ _____

Name of Person taking consent Date Signature

4. Interviewer administered questionnaire

Main Questionnaire

Prior to the start of the survey the main questionnaire (that is available in English) should be translated into the local language and then back translated by an independent lay (non-medical, non-nursing) translator into English. This back translation should be 1) checked at the local centre for consistency with the original AND 2) sent to the coordinating centre for checking. The coordinating centre will make appropriate suggestions for changes.

During the survey the main questionnaire should be administered in a quiet private room by a trained interviewer.

Before administering the questionnaire, each interviewer should become familiar with each question, coding and skips. It is important for interviewers to understand why a question has been asked and its meaning. The reliability of the data collected depends on how the questionnaire is administered by interviewers, and therefore it is essential that the questions are asked in the same way by different interviewers at different centres. The interviewers will ask the questions exactly as they are, using the exact wording and order, as written on the survey questionnaire, avoiding any hints or verbal clues.

The ideal interviewer, either clinical or non-clinical staff, should be **neutral and non-judgemental!** Never surprised or disappointed, never approving or disapproving, never feeling embarrassed about personal questions, never asking leading questions but **sticking to the exact wording and order** as on the survey questionnaire.

Possible difficulties during the interview should be identified and discussed during the training session.

Queries occurring during the study should be referred to the coordinating centre.

General instruction for standardised face to face interviewing

1. **Adhere to the questions' order and wording.**
2. Be as **neutral** as possible. In order to avoid interviewer bias, interviewers should not suggest answers or ask extra questions and should not give supplementary explanations to help people to respond. Verbal or non-verbal clues or hints should be avoided. Respondents should never feel that a certain answer is expected.
3. If an interviewer personally knows a participant, this person should be passed to another interviewer in order to ensure confidentiality and anonymity.
4. The interviewer should be prepared for unexpected interruptions of the interview due to unexpected situations (i.e. someone entering the room).
5. If the respondent refuses to answer a question, don't try to persuade reluctant responders too much. That may increase the respondent's bias.
6. Fill in the responses at the moment people gave them. Never leave it for later!
7. If the respondent re-addresses a previous question at a later stage of the interview, the interviewer should record the comments and note which question they are relating to.
8. Repeat clearly and slowly the question if the respondent does not understand it, using the same wording as it is written on the standard questionnaire.
9. If the respondent does not understand a particular word, the only response the interviewer can give is **"whatever that means to you"**. An alternative would be to ask the respondent to define the term he/she does not understand. If the given definition is good, the interviewer can say so and repeat the question. It is not a good idea for interviewers to give definitions or explanations on what a particular word means, as that may result in bias.
10. If the respondent does not understand the question even after it has been repeated, the answer will be coded as "No"

11. A “Don’t know” response should differentiate from a genuine uncertainty and other possible reasons the respondent may hide (i.e. lack of understanding the question, diplomatic refusal). Difficult to sense but a good idea would be to repeat the question.
12. If a respondent starts to comment on something else, the interviewer can bring the respondent back to the point of interest and gently explain that the comment can be discussed at the end of the interview.
13. Never interrupt respondents before they have finished speaking. Allow enough time for response.
14. Follow the skips where indicated. It is important to jump over inapplicable questions and this will also save time.
15. Read questions clearly, at an appropriate volume and speed, and make sure that the responses are accurately recorded.
16. If a respondent is not sure or does not know what to answer, tick “No”.
17. In case of a refusal, try to encourage participation by being friendly and polite, offer to start the questionnaire and see how things go, gain trust by giving more of an explanation about the study goals, ethical approval and the time required for the interview.
18. If a respondent does not want to reveal personal information such as date of birth, the interviewer should re-confirm that this is confidential and the research goal is to assess the proportion of people suffering with allergic diseases in general and not necessarily to look at individuals’ data. If the respondents remain reluctant, then their wishes should be respected.
19. Verbatim notes can be made on the respondents’ comments. However, respondents should be reminded that they should answer with “Yes” or “No” only.

All interviewers should remember the golden rules

1. Read the questions exactly as they are written on the questionnaire.
2. Do not give any extra-explanations or verbal or non-verbal clues even if the respondent is asking you or does not understand the question.
3. Emphasize the words written in bold and underlined.
4. If the respondent is unsure of the answer please tick 'NO'.

5. Exhaled Nitric Oxide measures

Exhaled nitric oxide measures should be made after completion of the 'Getting ready for FENO, spirometry, reversibility and bioimpedence' questionnaire.

Exhaled nitric oxide levels should be measured before other spirometric assessment and before skin prick testing.

The NIOX MINO will be used to make **one** measure of F_eNO

For one hour prior to measurement participants should refrain from
- Smoking for one hour
- Eating or drinking for one hour
- Strenuous exercise

The NIOX MINO should be turned on at least 15 minutes prior to use, and set for a 10 second inhalation. At all times the NIOX Mino should be kept away from
- mobile phones, computers and other electromagnetic forces
- direct heat
- drafts

The mouthpiece is inserted. The procedure should be explained to the participant.

Measurements are made in the sitting position. A mirror should be placed on a nearby table such that participants can see the image of the screen. This will help them know if they are exhaling at the correct speed.

Participants are asked to
- empty their lungs through a single long exhalation
- place their lips around the mouthpiece and take a deep breath until they reach total lung capacity
- 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) on the NIOX MINO. The NIOX MINO will indicate when 10 seconds is complete.

FeNO is measured at the plateau of expiration and given in parts per billion. This figure 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.

The training video should be seen by all fieldworkers as part of their training.
http://www.aerocrine.com/en/niox-mino/Videowindow.html

6. Measurement of height, weight, waist/hip circumference and bioelectrical impedence

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

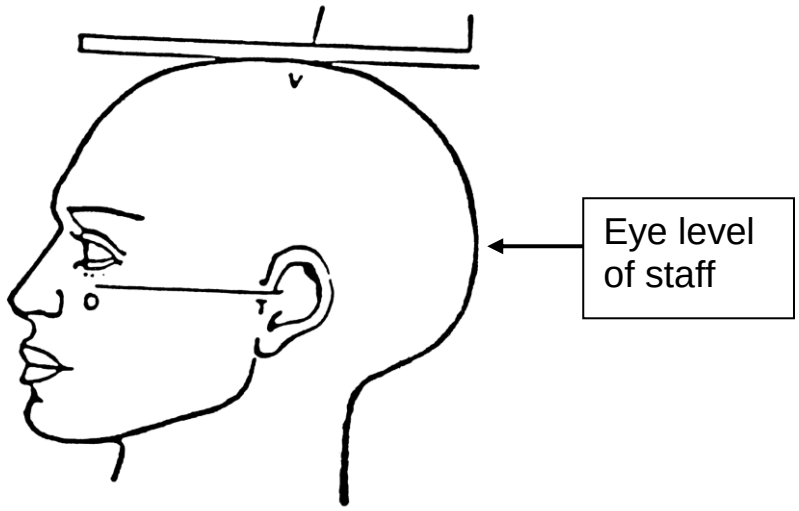
1. Ask the participant to remove shoes, hat and bulky clothing such as coats and sweaters. You may need to ask some participants to adjust hairstyles or remove hair accessories that may interfere with measurement.

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.

<u>Bioimpedence</u>

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

1) new version of the BodyStat 1500 MDD (**<u>NOT</u>** 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

1) Women who are pregnant
2) Those who have a pacemaker or defibrillator
3) 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

1) 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
2) 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

1) 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
2) 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.

7. Skin prick testing

Skin prick testing will be carried out using skin testing reagents and standard lancets available from ALK-ABELLO.

Twelve allergens will be tested in all centres plus a positive and negative control.

Each subject will be skin tested using the following panel of allergens at the stated concentration. In centres where for local reasons it is impossible to comply with this protocol the details of the deviation form protocol should be clearly stated on the Centre Equipment List Inventory.

Timothy Grass	10 HEP
Ragweed	1:100 W/V
D. pteronyssinus	10 HEP
Cat	10 HEP
Birch	10 HEP
Blatella (German Cockroach)	1:100 W/V
Olive	30HEP
Alternaria	1:20 W/V
Dog	10 HEP
Cladosporium	1:20 W/V
Parietaria	10 HEP
D. farinae	10 HEP
Positive Control	10mg/mL histamine
Negative Control	0.9% saline

Equipment
Skin test solutions must be stored at +4^{o}C when not in use.

Other necessary equipment:

- skin test grid for application of tests
- lancets
- tissues
- sink, soap, hand towels
- clinical gloves
- sharps bin
- transparent scotch 3M tape at least 25 mm wide
- ball-point pen or fine felt tip pen
- timer with alarm.
- antihistamine cream
- Skin prick test results sheet

A template for the skin test grid is provided. This can be printed onto transparent paper (such as overhead projection paper) and then the grids cut out as required. The same grid can be used for several different participants, so long as they are cleaned with water and detergent and then wiped with alcohol between uses.

Method

Skin prick testing should be carried out after measurement of exhaled NO.

Fieldworkers should firstly ask question 1 on the skin prick test data collection sheet, recording the time of last use of antihistamine medication

Trained study staff should carry out the skin testing according to the following instructions:

Wash hands and apply clinical gloves

1. Place a clean test grid on volar surface of the forearm and fix with transparent or surgical tape. Mark the orientation of the grid on the subject's arm (e.g. mark top and bottom of grid).

2. Place a small drop of skin testing solution in the centre of each grid square. (Apply the skin test allergens in the same order during each test.)

3. Unwrap a lancet according to manufacturer's instructions. Hold the lancet at 90^{o} to the skin and with the forefinger press through the drop against the skin for at least 1 second. Very little pressure is required. A small impression may be briefly visible on the skin. The skin should not be broken to the extent that blood is drawn. Always apply the same pressure.

4. Remove the lancet with an upward motion and discard into a sharps container.

5. Change the lancet skin puncture device between each allergen test sites to avoid false positive results.

6. Remove the skin test grid. Blot any excess solution with tissues taking care not to cross-contaminate the tests.

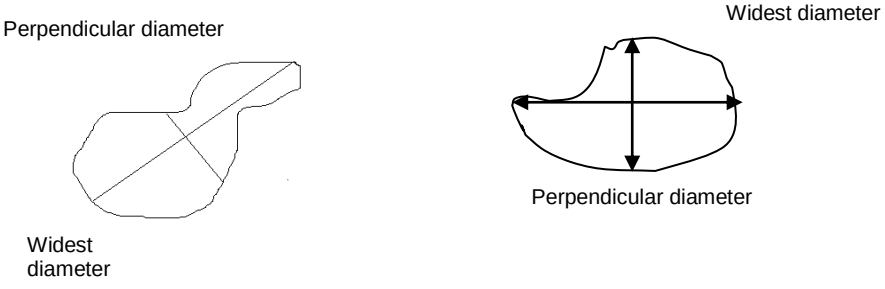
7. Set the timer alarm and read the results after 15 minutes. During this wait, review the self administered questionnaires.

8. To record the results of the skin prick test draw around the perimeter of each of the wheals with a ballpoint pen or fine felt-tip pen. Always draw in the same order as the application of the tests.

9. Press a strip of transparent Scotch tape against the skin and transfer the prints to the grid on the results sheet. The transfer should always be placed at the same orientation marked on the grid.

10. From the transfer first measure the weal diameter (mm) at it's widest. The second diameter is called the 'perpendicular diameter'. This should be drawn at 90^{O} to the first diameter and **at the mid-point** of the first diameter. The second diameter may therefore not necessarily be at a wide point on the weal. Record both diameters to the nearest whole millimetre on the results sheet.

Figure1. Measurement of a skin prick weal.



11. When rounding to the nearest whole millimetre use the convention: 1.0-1.4 mm round down (1 mm), 1.5- 1.9 mm round up (2 mm).

12. If the participant has itchy and uncomfortable wheals after testing, reassure them that they will normally resolve after ½ hour and apply antihistamine cream as required.

Other measures of skin prick test reactivity
Computer software that can scan the skin prick test record sheet are available. All centres should preserve their skin prick testing sheets so that this approach to wheal measurement can be used at an unspecified point in the future.

Training
Project study staff must be trained to perform skin tests consistently and in a standardised manner. Before starting the study, staff should perform two histamine skin tests on each of 10 participants (total 20 tests done by each trainee).

The results can be recorded on the allergy skin test training sheet supplied.

Participants can be tested with allergens if they wish, but only the histamine weal results need be recorded for the purpose of the training.

Trained staff should have a coefficient of variation (CV) of less than 30%.
The coefficient of variation of each staff member is carried out as follows:

Calculate the log to base e of each mean weal diameter recorded in mm.
If there are exactly two skin tests carried out on each participant:
Use the following formula to calculate the CV:

$$CV = \sqrt{\frac{\Sigma(d^2/2}{n}} \times 100$$

where
d = difference between two $\log_e$ values for each participant
n = number of participants

Use the coefficient of variation calculation sheet provided in Appendix 14

If there are not exactly two skin tests for each participant:
A between participant one-way analysis of variance can be carried out using a suitable computer program or calculator. Obtain the residual mean square, take the square root and multiply by 100 to obtain the CV (%).

Trainees should also administer the entire panel of allergens on five occasions and record them on a skin prick test result sheet (as per Method sub-chapter) before starting data collection with study participants. Document that this training has taken place.

Centre ID

Skin Prick Allergy tests

No Yes

1. Have you taken any antihistamine tablets in the last 24 hours?

If yes please given the name of the drug and the time last taken?

CODE

1.1Name of antihistamine tablet_____

1.2 How many hours ago was it taken?

Tim grass	Ragweed
D pter	Cat
Birch	Blattella
Olive	Alternaria
Dog	Cladosporium
Parietaria	D farinae
+ve	-ve

Tim Grass — 1st diam 2nd diam

Ragweed — 1st diam 2nd diam

D Pter — 1st diam 2nd diam

Cat — 1st diam 2nd diam

Birch — 1st diam 2nd diam

Blattella — 1st diam 2nd diam

Olive — 1st diam 2nd diam

Alternaria — 1st diam 2nd diam

Dog — 1st diam 2nd diam

Cladosporium — 1st diam 2nd diam

Parietaria — 1st diam 2nd diam

D farinae — 1st diam 2nd diam

+ve — 1st diam 2nd diam

-ve — 1st diam 2nd diam

Skin Prick Test template

Tim Grass	Ragweed
Der pter	Cat
Birch	Blattella
Olive	Alternaria
Dog	Cladosporium
Parietaria	Der farinae
+ve	-ve

Allergy Skin Test Training Sheet

Fieldworker identifier

Carry out two histamine skin prick tests on each participant. Record diameters to the nearest mm.

Participant name / number: ________________________________ Date:

TEST 1 TEST 2

1st diam 2nd diam Mean 1st diam 2nd diam Mean

Participant name / number :________________________________ Date:

TEST 1 TEST 2

1st diam 2nd diam Mean 1st diam 2nd diam Mean

Participant name / number: ________________________________ Date:

TEST 1 TEST 2

1st diam 2nd diam Mean 1st diam 2nd diam Mean

Participant name / number: ________________________________ Date:

TEST 1 TEST 2

1st diam 2nd diam Mean 1st diam 2nd diam Mean

Participant name / number: ________________________________ Date:

TEST 1 TEST 2

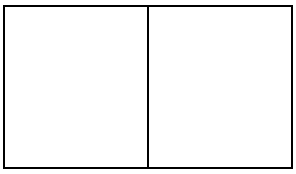
1st diam 2nd diam Mean 1st diam 2nd diam Mean

Participant name / number: ________________________________ Date:

TEST 1 TEST 2

1st diam 2nd diam Mean st diam 2nd diam Mean

Participant name / number: _____ Date:



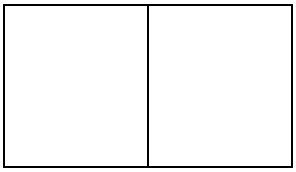
TEST 1

1st diam 2nd diam Mean

TEST 2

1st diam 2nd diam Mean

Participant name / number: _____ Date:



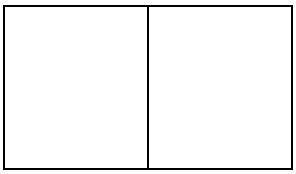
TEST 1

1st diam 2nd diam Mean

TEST 2

1st diam 2nd diam Mean

Participant name / number: _____ Date:



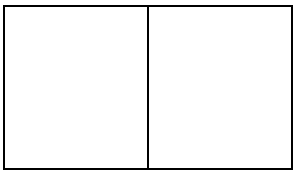
TEST 1

1st diam 2nd diam Mean

TEST 2

1st diam 2nd diam Mean

Participant name / number: _____ Date:



TEST 1

1st diam 2nd diam Mean

TEST 2

1st diam 2nd diam Mean

Calculation of CV

If 2 Tests Carried Out On Each Participant:

	Mean diam TEST 1 (A)	Mean diam TEST 2 (B)	Log_e (A)	Log_e (B)	d	d^2	$d^2/2$
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
				$\sum d^2/2$			
				$\frac{\sum d^2/2}{n}$			

Coefficient of variation CV = $\sqrt{\frac{\Sigma(d^2/2)}{n}}$ x 100 =

where diam= diameter
$\log_e$ = log to base e
d = $\log_e$ (mean diam 1) - $\log_e$ (mean diam 2)
n = number of participants

Reference: Chinn S. The assessment of methods of measurement. Statistics in Medicine 1990; 9:351-62

8. 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:

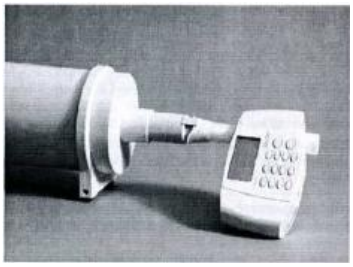
Sink for hand washing, soap and hand towels
Containers of:
clean mouthpieces (Spirettes)
nose-clips
Containers to collect:
used Spirettes
used nose clips
Box of tissues

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™ 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.

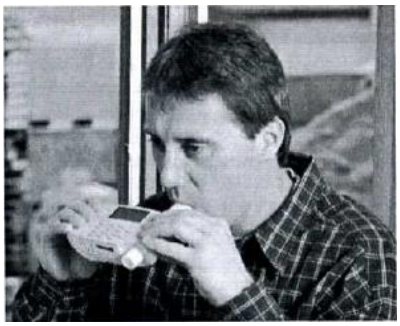
After instructing the participant about the procedure for pulmonary function testing the following procedures (outlined in sections 5.2 to 5.4 of the ndd EasyGuide™ users' manual) should be followed. This initial series of manoeuvres is performed **BEFORE** administering the bronchodilator.

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

1. The fieldworker shakes the inhaler and places it on the spacer
2. The participant is asked to exhale fully, tip their chin up slightly and place their lips around the spacer.
3. 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
4. The participant inhales slowly and deeply to total lung capacity and then hold their breath for 10 seconds
5. 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

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 anonnymised 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

9. Venesection

The aim is to collect 20mL of 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.

Bottle	Colour	Size	Number of inversions to mix	Purpose	Overview of handling	Storage at -20°C
Gel Serum Separator BD SST™	Gold	2x 7mLs	5	IgE testing	Allow to clot for 60 minutes. Centrifuge for 15 minutes at 3000 rpm & prepare aliquots	1 aliquots of 2.5mL in 3.5 mL Starstedt tube with screw cap Remaining aliquots of 1mL Into storage boxes
EDTA	Lavender	6mLS	10	Extraction of DNA and other genetic material	-	Store directly

2.5mLs of serum will be sent at -20°C for measurement of total IgE, serum specific IgE for environmental allergens and for food allergens.

Remaining samples will be used for further as yet unspecified research. Further ethical approval will be required from Research Ethics Committees when the precise nature of this future research is agreed.

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,

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.

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.

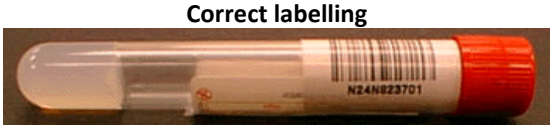
Remove bottle from holder, keeping needle situated in the vein and continue to fill the blood bottles in correct order of draw. **Mix each blood tube as required before inserting a new tube.** The exchange of vacutainers should be smooth and the final blood tube removed prior to the needle being withdrawn 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.

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

Correct labelling



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 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

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.

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**

Centrifugation Speed: 3000 rpm Centrifugation Time: **15 minutes**

Freezer Temperature: **-20 °c** 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

The 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.

10. Urine collection

The aim is to collect 18 mLs of urine from each participant and from this to prepare three aliquots of urine in a vacutainer for long term storage at -20^{o}C.

Participants are provided with a wide mouthed sterile container. About 20 mLs of a mid-stream specimen of urine are collected directly into this container and stored in a fridge for no longer than 24 hours prior to preparation of further aliquots for storage.

Six mls of urine is drawn into a plastic syringe and passed through 0.45mm filter paper (to remove cells) into a storage vial. Samples are then labelled with centre and id number and stored at at least -20^{o}C

11 Food Frequency questionnaire

Standard Operational Procedure (SOP) for translation of the Food frequency questionnaire (FFQ) for ECRHS III

Prior to starting the survey each centre should discuss translation of the FFQ with coordinating centre staff. The questionnaire that will be used is NOT exactly the same as the GA2LEN FFQ or the BOLD FFQ – although there are considerable similarities.

All centres should nominate a single person to be responsible for the nutritional component of the survey and send their name and a contact details to Vanessa Garcia-Larsen (VGL) (v.garcialarsen@imperial.ac.uk) Countries that have already a translated version of the GA^{2}LEN or BOLD FFQ will be provided with a fully translated version of the ECRHS III FFQ. These countries are Sweden and Denmark.

Centres that do not have a GA^{2}LEN FFQ or BOLD FFQ already translated will translate the FFQ into the local language following the Translation SOP according to the WHO guidelines.
Briefly, these are the 3 steps that need to be followed – please refer to Figure 1 for the full details of the translation SOP.

1) Questions to be translated into the local language by a native speaker of the local language, with a brief report and the problems encountered,
2) Back translated by ANOTHER person who has not previously seen the original FFQ in English,
3) This back translation, with the brief report is reviewed by the coordinating centre – Vanessa Garcia-Larsen

VGL will approve the final version before it can be used

The FFQ should be sent to participants prior to the clinic visits (so that they can complete before they attend) or may be self-completed in the clinic. Whatever is done it is essential that the questionnaire is **CHECKED** for completeness of the answers by the field worker. If some questions have been omitted in error and answers are not given the fieldworker should return the questionnaire to the participant and ask them to complete it.

SOP is used to ensure that all participant centres collect the same data.

The English FFQ is the final version sent to the Centres.

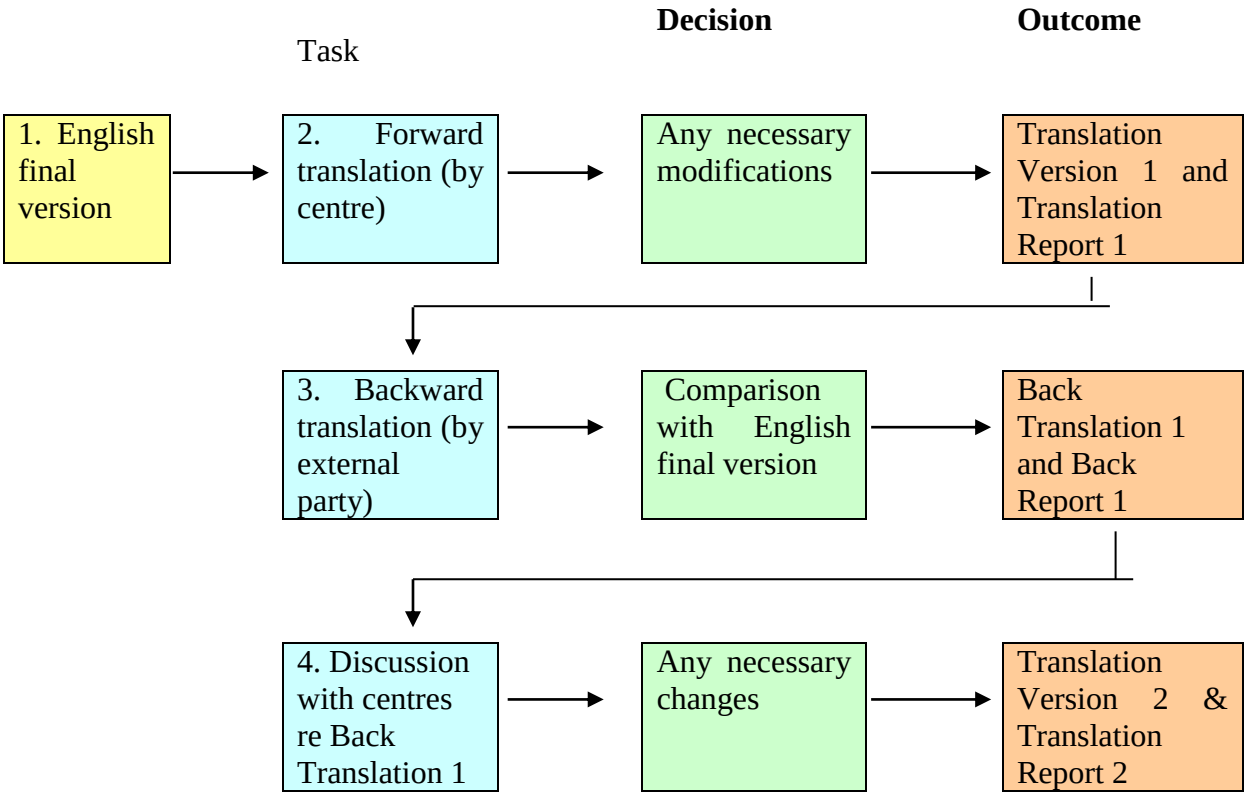
Forward translation into local language should follow the following procedure (Figure 1).

1) Recruit a local translator (native target language and bilingual UK English), this could be a member of the study team.
2) The translator produces 'Translation version1'. This should be a conceptual equivalent of the English final version document, in colloquial language and easy to understand.
3) If local centre staff (with or without the translator) assess that 'Translation version 1' needs modification then 'Translation version 1.1' incorporating any changes should be produced.
4) Centre staff should produce Translation report 1 and send to the co-ordinating centre (London) Translation version1 and (if appropriate) Translation version 1.1. These can be sent to Vanessa Garcia-Larsen (v.garcialarsen@imperial.ac.uk)
5) The translated documents should be in editable electronic formats compatible with Microsoft Word. The translation report 1 should describe how the translation was produced and outline (question by question) any issues that have arisen so far.

Once the "Translation Version 1" has been produced, centres are asked to arrange for back translations to be undertaken locally. It is very reasonable that a member of the study team undertake the forward translation, however, the back translation must be undertaken by a different

person and someone completely unrelated to the work of the centre. They should not have specialist knowledge of the survey work.

Figure 1: Flow chart of translation process



The back translator should not have access to the original English final version while producing the translated questionnaire in English or 'Back translation 1'. Centres are asked to compare Back translation 1 with the English final version and produce Back report 1 identifying any misunderstandings or inaccuracies in Translation version1 (or Translation version1.1). Back report 1 should also state the main occupation of the back translator. Please transfer Back translation 1 and Back report 1 to v.garcialarsen@imperial.ac.uk

Co-ordinating centre staff will confer with centre staff (if possible the forward translator) to negotiate changes to Translation version 1 (or 1.1).

Following these discussions the centre should produce Translation version 2 and Translation report 2. Translation report 2 should be in English and detail the changes made to Translation version 1 with the preferred target language expressions and their English equivalents.

Following submission of the dietary questionnaires as "Translation version 2", the FFQ can be used in each participant centre.

12 Self completed questionnaires

12.1 Short International Physical Activity Questionnaire

Each participants should complete the Short Physical Activity Questionnaire. This is available from the following website and comprises 7 questions.

A version of this questionnaire is available in most languages form the following website http://www.ipaq.ki.se/downloads.htm. Please ensure you download the **SHORT format** (only 7 questions)

The following coding instructions should be followed

For question 1 and 3 and 5
Answers can be 0 - (no days doing activity as specified) or 1-7 (depending number of days)

For questions 2a, 4a, 6a and 7a
Answers can be from 0 to 20
'Don't know' 'not sure' should be entered as '88' if 'best guess' not possible

For questions 2b, 4b, 6b and 7b
Answers can be from 0 to 59
'Don't know' 'not sure' should be entered as '88' if 'best guess' not possible

NB If you provide the answer in hours DO NOT provide the answer also in minutes
(That is, if someone spends one hour per day doing vigorous physical activity DO NOT provide answer '01' to 2a and answer '60' to 2b. In this circumstance provide answer '01' to 2a and '0' to 2b)

12.2 Asthma control test

The asthma control test should be completed by any person who has answered yes to 'Have you ever had asthma?' (q 15) in the main questionnaire.

This is a short five item questionnaire that is governed by copyright and cannot be included here. It is available on the website at http://www.asthmacontroltest.com/

Please contact the coordinating centre to see if they have a pdf version of the questionnaire authorised by Glaxo for use in your country.

12.3 Juniper Asthma Quality of Life Questionnaire

The asthma quality of life questionnaire should be completed by any person who has answered yes to 'Have you ever had asthma?' (q 15) in the main questionnaire.

This questionnaire is governed by copyright and is not included here. Each PI will receive a hard copy of this questionnaire from Dr Benedicte Leyneart.

12.4 Generic Quality of Life Questionnaire

The generic quality of life questionnaire should be completed by all participants. It will be the SF-36 and centre should use the same version as used in ECRHS II (Please note that in ECRHS II two additional questions on long standing illness were included. However as this information is now collected in the main questionnaire these two questions should not be included).

This questionnaire is governed by copyright and is not included here. Each PI will receive further specifications from Dr Benedicte Leyneart.

12.5 Sleep questionnaire

Two forms of the sleep questionnaire will be used as part of the ECRHS III protocol.

Centres that have previously collected information on sleep from the cohort (Scandinavian centres and Belgium) will use one version (coordinated by Prof Janson in Uppsala).

Centres that have NOT previously collected information on sleep will complete another SLEEP questionnaire.

12.6 Sunlight questionnaire

A short questionnaire asking about exposure to sunlight will be self completed by participants.

13. Nasal lining fluid (UK only)

A small amount of the fluid within the nose will be absorbed on to a small strip of synthetic absorptive matrix (SAM).

Participants are aksed to sit down and title their head backwards. A strip of SAM will be placed inside the nose and over the inferior turbinate using disposable forceps. Participants are asked to press the outer edge of the nose towards the septum using their finger and to hold it there for 2 minutes. The SAM is removed and placed into a small Starstedt tube and frozen at -20^{o}C until later analysis.

Samples will be stored for up to 2 years. Nasal lining fluid will be obtained from the Sam by centrugation and cytokines assessed using the Meso-Scale Discovery System at Imperial College.