MeDALL SOPs

WP3 and 4

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MeDALL

WP4 Standard Operating Procedure
Height and Weight

The order of physical examinations applied by the cohorts at the study centers will be:

1) Measure height and weight
2) Atopic dermatitis (screening and, if necessary, severity rating)
3) FeNO (**WP4 only**)
3) Spirometry
4) Blood drawing

Premises for measurement

- Closed and protected room for physical examination

- Comfortable room temperature

- The choice of the measurement devices for height (stadiometer) and weight (floor scale) will be made by each cohort individually

Further equipment
- Step stool
- Chair (or something alike to put the participant's clothes on)
- Paper backing (hygienic sheet for platform of stadiometer and scale)

Preparation for measurement

- Renewing of paper backing on platform
- The measurement takes place in light clothing
- Shoes, thick socks, jackets, pullovers, jewelry, contents of the pockets and anything heavy have to be removed
- The participant is allowed to wear light trousers, socks and undershirt or t-shirt

Weight

Set up of the scale

- Scale has to be placed on solid ground (no carpet)
- Check horizontal orientation of scale
- Scale should be calibrated with 20 kg and 40kg every 3 months; electronic scale with calibration class III every 2 years

Measurement

- Place paper backing on scale
- Turn scale on
- Participant stands centrically on scale
- Perform measurement
- Results are noted on the measurement log sheet with an accuracy of ± 100g MeDALL WP4 SOP Weight and Height May, 2011 3

Height

Measurement

- Place paper backing on floor plate
- The participant stands upright and centrically on the floor plate, the back turning towards the meter
- The participant touches the meter with:
  - Heals
  - Buttocks
  - Back
  - Occiput

-.Place the slider above the participants head and bring orbital margin and the tragion of the participant in a horizontal line (see horizontal line in figure 1).

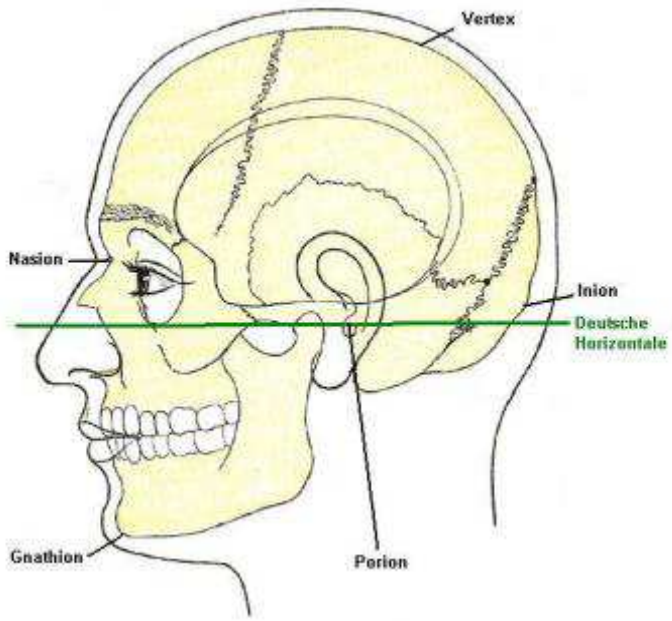


Figure1. http://de.academic.ru/pictures/dewiki/71/
Gray_Surface-head.jpg

- The participant's hair is pressed flat by the slider
- The participant is instructed to stay in an upright position and to breathe normally during measurement
- Results are noted on the measurement log sheet with an accuracy of ± 0.1cm

MeDALL

Standard Operating Procedures
Atopic Dermatitis

Screening for Atopic Dermatitis

The Hywel William's Criteria are used as screening instrument for atopic dermatitis (3 minutes, questionnaire is included in the Appendix).

(The following sections "Basic Rules" and "Clarification of Questions" derive from the United Kingdom Working Party homepage: http://www.nottingham.ac.uk/dermatology/eczema/contents.html)

Basic Rules

- If a problem arises with a question response, the following rules should be followed:
- The question is repeated exactly as it is written, emphasizing wording (e.g. "IN THE LAST YEAR") where there is ambiguity or misunderstanding.
- The subject is reminded that he or she should try to answer "yes" or "no" to each of the questions where these are the given answers.
- If an answer of yes or no is required and the subject does not understand the question even when repeated, the answer is coded as "no".
- For some of the questions an explanation may be given to the subject and instructions for these questions are provided below.

Clarification of Questions

- **Q1:** This refers to the presence/absence of itchy skin condition (the only necessary diagnostic feature) and the time period for reporting symptoms. It is important for the interviewer to emphasize the time period for reporting symptoms by repetition if necessary. We recommend retaining this question stemming as it enables one year period and point prevalence to be recorded.
- **Q2:** Age of onset: the respondent's best guess may be accepted. For surveys conducted in those aged 16 and over, the respondent's parental recall of age of onset is preferable to respondent recall.

If visible flexural dermatitis/itchy skin condition (Question 3 for SCORAD and POEM) is rated with "Yes"
→ the objective SCORAD or the POEM is used for severity rating of eczema.

SCORing Atopic Dermatitis (SCORAD)

The objective SCORAD rates symptom severity of dermatitis on two levels (evaluation sheet in included in the Appendix):

- (A) extend of symptoms
- (B) intensity of symptoms.

(The following sections "Analyses of surface involvement" and "Grading of Intensity" derive from the SCORAD homepage: http://adserver.sante.univ-nantes.fr/Scorad_Course/How.html)

The physical examination

- For the physical examination the participants should get undressed (underwear remains on).

(A) Analysis of surface involvement

- During the clinical examination, draw the involved areas on the evaluation sheet.
- Then calculate the proportion of involved surface area segment by segment (see this Figure 1). For participants >20 months of age, the surfaces are counted as the following proportions:

  Front
  - Face 4.5%
  - Upper Limbs 9%
  - Trunk 18%
  - Hands 1% per palm (according to the European task force on AD), so 2%
  - Lower Limbs 18%
  - Genitals 1%

  Back
  - Head 4.5%
  - Upper Limbs 9%
  - Trunk 18%
  - Lower Limbs 18%

- Total the results for all segments. (If every segment would be rated as 100% involved, this would result in a maximum score of surface involvement of 102%).

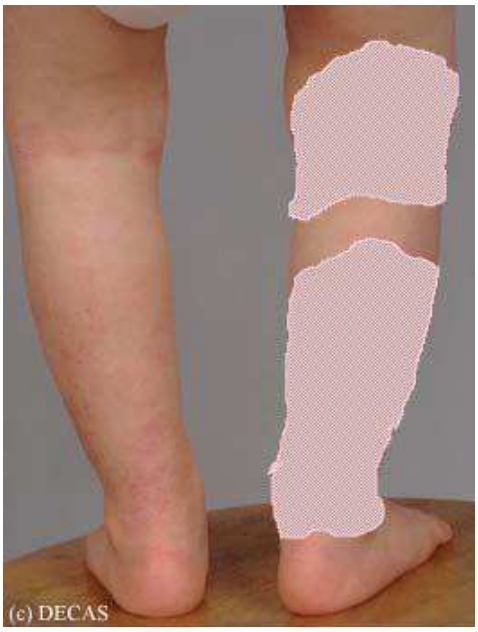


Figure 1. On this example, 3/4th of the right lower limb corresponds to a score of about 7/9 (Ref: http://adserver.sante.univ-nantes.fr/Scorad_Course/Example.html).

Training for section (A) is offered on the following website: http://adserver.sante.univ-nantes.fr/Scorad_Course/Case_2_spread.html.

(B) Grading of intensity

- Choose the representative area: In patients with areas varying in severity, a representative area of mean involvement is chosen. *Dryness should be evaluated in a remote area of noninflammatory skin.*
- Identify the elementary lesions (see Figure 2).

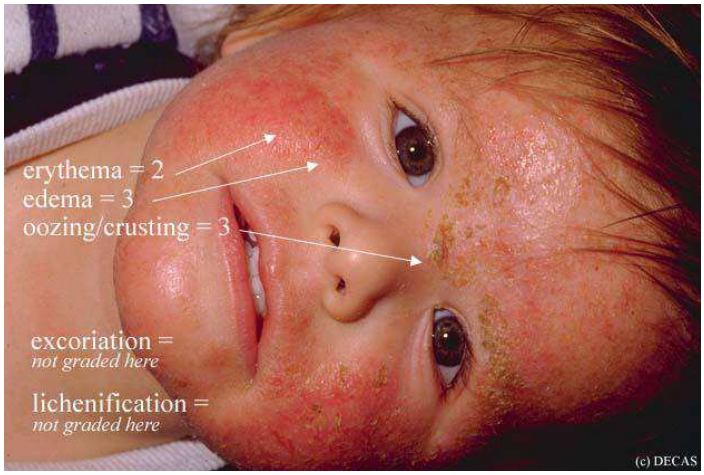


Figure 2. Identification of each item is the first step followed by evaluation of intensity on a scale of 0 to 3. The total for the intensity criteria is based on a maximum of 18. (Ref: http://adserver.sante.univ-nantes.fr/Scorad_Course/Identify.html) *Training for section (B) is offered on the following website: http://adserver.sante.univ-nantes.fr/Scorad_Course/Case_2_intensity.html.*

Scoring
Skin "lesions are not included in the scoring if they make less than 3% of the body surface"

The score is calculated by the following formula:
- A/5 + 7B/2 (**maximum score 83.4**)*

Alternatively, the SCORAD homepage offers the calculation of the score: http://adserver.sante.univ-nantes.fr/Compute.html.

*Concerning the computation of the SCORAD, there are two rules depending on the age of the patient:

- In children **aged<2 years, genitals and palms should not be considered**. ***The sum of body areas is 101.***
- In children a**ged >2 two years and adults, genitals (1) and palms (1+1) should be considered**. ***The sum of body areas is 102***.

Totals are not 100.This is explained by the fact that the SCORAD is based on "the rule of nine"; in other words, the A component of the score is the sum of multiples of nine and of three points representing genitals and palms.

However, since we will only measure the objective SCORAD, the grading should be the following:

- < 15 Mild
- 15-40 Moderate
- >40 Severe

Patient Oriented Eczema Measure (POEM)

If visible flexural dermatitis is rated with “Yes”, the POEM might be alternatively applied instead of the SCORAD.
While the SCORAD is an observer-based severity rating, the POEM is a patient-based and therefore self-assessed questionnaire on symptom severity of eczema (3 minutes, questionnaire in included in the Appendix).

Appendix

Hywel William’s Criteria IF YOU USE THE SCORAD FOR SEVERITY RATING

1. IN THE LAST YEAR, have you had an ITCHY skin condition - by *itchy* we mean scratching or rubbing the skin? YES[ ] NO[ ]

NO: Please, skip the following questions and proceed with the FeNO measurement.
YES: Please proceed with question 2.

2. How old have you been when this skin condition began?
Under 2 [ ] 2 to 5 [ ] 6 to 10 [ ] Over 10 [ ]

Physical examination by investigator:
3. Is there visible dermatitis today?
YES[ ] NO[ ]

NO: Please, skip the SCORAD and proceed with the FeNO measurement.
YES: Please proceed with the SCORAD.

Hywel William’s Criteria IF YOU USE THE POEM FOR SEVERITY RATING

1. IN THE LAST YEAR, have you had an ITCHY skin condition - by *itchy* we mean scratching or rubbing the skin? YES[ ] NO[ ]

NO: Please, skip the following questions and proceed with the FeNO measurement.
YES: Please proceed with question 2.

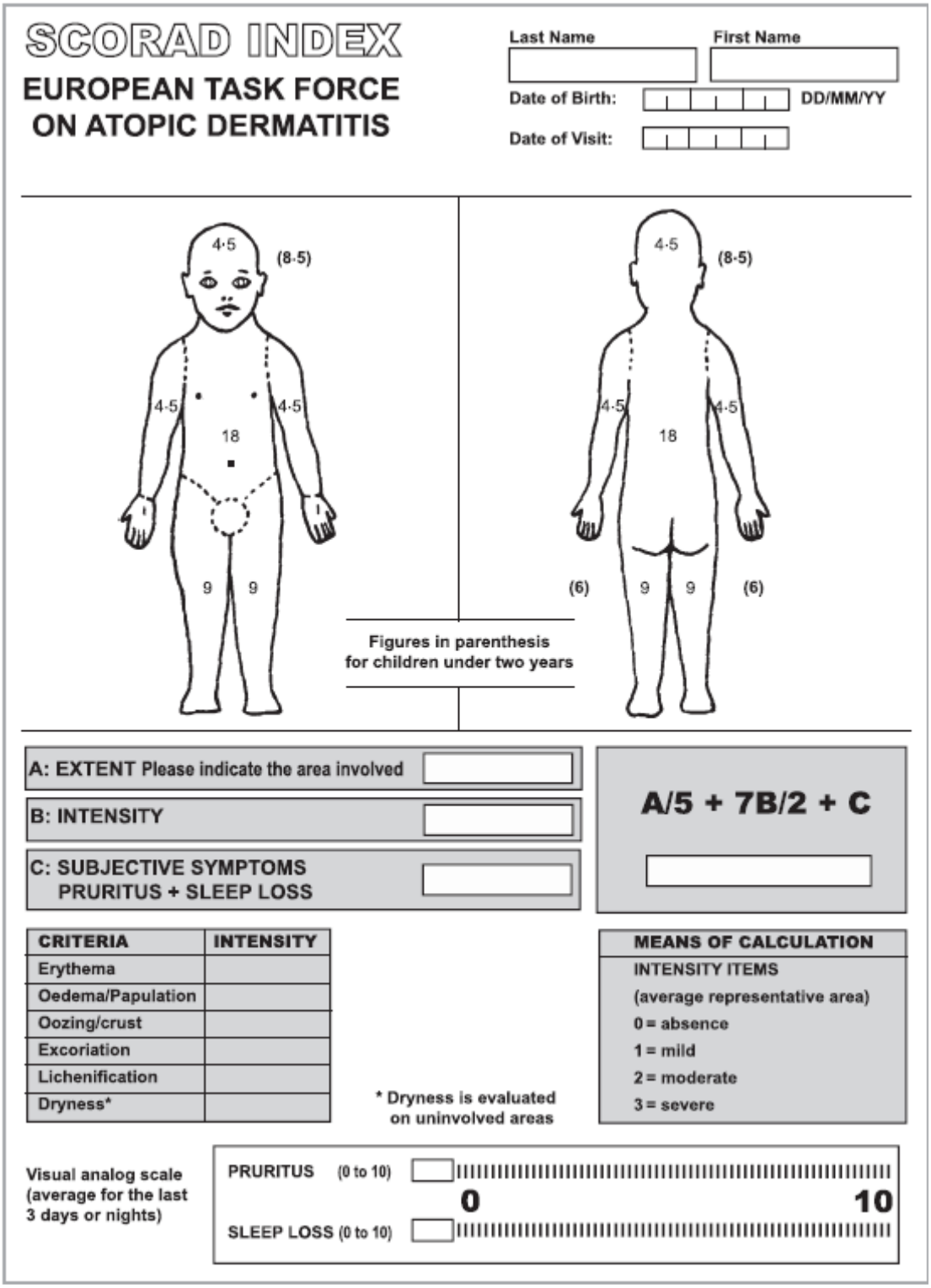
2. How old have you been when this skin condition began?
Under 2 [ ] 2 to 5 [ ] 6 to 10 [ ] Over 10 [ ]

3. Have you had this ITCHY skin condition in the LAST WEEK? YES[ ] NO[ ]
NO: Please, skip the POEM and proceed with the FeNO measurement.
YES: Please proceed with the POEM.

Definition: One or more patches of dermatitis affecting ankles, fronts of elbows, sides or front of the neck, or around the ears or eyes. Dermatitis may be defined as “poorly demarcated erythema with surface change”. Surface change can mean scaling, crusting, vesicles or lichenification.

The photographic protocol for observers is a reference manual for recording signs of visible flexural dermatitis and can be downloaded from the following website: http://www.nottingham.ac.uk/dermatology/eczema/Section3-2.html.

Objective SCORAD evaluation sheet (Please note that subjective measurements (C) will not be measured. Only extent (A) and intensity (B) will be measured with additional 10 points for disfiguring/functional lesions if any).



Ref: European Task Force on Atopic Dermatitis. Severity scoring of atopic dermatitis: the SCORAD Index (consensus report of the European Task Force on Atopic Dermatitis) (1993). *Dermatology* 186:23–31.

Patient-Orientated Eczema Measure (POEM)
Name: Date:
Date of birth:
Total POEM score (maximum 28):

Please circle one response for each of the seven questions below. Young children should complete the questionnaire with the help of their parents. Please leave blank any questions you feel unable to answer.

1. Over the last week, on how many days has your / your child's skin been itchy because of the eczema?
No days/ 1-2 days/ 3-4 days/ 5-6 days/ Every day

2. Over the last week, on how many nights has your / your child's sleep been disturbed because of the eczema?
No days/ 1-2 days/ 3-4 days/ 5-6 days/ Every day

3. Over the last week, on how many days has your / your child's skin been bleeding because of the eczema?
No days/ 1-2 days/ 3-4 days/ 5-6 days/ Every day

4. Over the last week, on how many days has your / your child's skin been weeping or oozing clear fluid because of the eczema?
No days/ 1-2 days/ 3-4 days/ 5-6 days/ Every day

5. Over the last week, on how many days has your / your child's skin been cracked because of the eczema?
No days/ 1-2 days/ 3-4 days/ 5-6 days/ Every day

6. Over the last week, on how many days has your / your child's skin been flaking off because of the eczema?
No days/ 1-2 days/ 3-4 days/ 5-6 days/ Every day

7. Over the last week, on how many days has your / your child's skin felt dry or rough because of the eczema?
No days/ 1-2 days/ 3-4 days/ 5-6 days/ Every day

MeDALL

Standard Operating Procedure
FeNO

ERS/ATS guidelines

All cohorts apply the ERS/ATS guidelines for measurement of FeNO.

The following descriptions are selected sections from the ERS/ATS guideline:
ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide (2005). *Am J Respir Crit Care Med* 171: 912–930.

General Principles Regarding Exhaled NO Measurement

- Because spirometric maneuvers have been shown to transiently reduce exhaled NO levels, it is recommended that **NO analysis is performed before spirometry**. The same stipulation applies to other taxing respiratory maneuvers, unless these can be shown not to influence exhaled NO. The FeNO maneuver itself and body plethysmography do not appear to affect plateau exhaled NO levels.
- **Breath-hold** results in NO accumulation in the nasal cavity, lower airway, probably in the oropharynx, which causes NO peaks in the exhalation profiles of NO versus time and is **discouraged in the standardized techniques**.
- Patients should refrain from eating and drinking for 1 hour before exhaled NO measurement. It is possible that a mouthwash may reduce the effect of nitrate-containing foods. **Recent food intake and drinking of the participant will be assessed by questionnaire.**
- It is uncertain whether measurements need to be standardized for time of day. Where possible, serial **NO measurements in the same period of the day should be performed** and the **time of day should always be recorded**.
- Subjects should not smoke in the hour before measurements, and **short- and long-term active and passive smoking history should be recorded**.
- FeNO measurements should be deferred until recovery from upper and lower respiratory tract viral infections if possible or the infection should be recorded in the chart.
- The potential effect of drugs on NO cannot be excluded, and all current medication and time administered should be recorded.

→ *Please, see the attachment for questions to be used before physical examination.*

Recommended Technique for Online Adult Exhaled NO Measurement

- **Mobile phones should be switched off during measurement.**
- **For each maneuver the number of the maneuvers will be recorded.**

Inspired gas source

- The use of NO free air (containing < 5 ppb) for inhalation is preferable.
- In all studies, it is advisable to record ambient levels of NO.

Inhalation Phase

- The patient should be seated comfortably, with the mouthpiece at the proper height and position.
- A nose clip should not be used. However, if a subject cannot avoid nasal inspiration (seen as an early expiratory peak) or nasal exhalation, a nose clip may be used.

- The patient inserts a mouthpiece and **inhales over 2 to 3 seconds through the mouth to total lung capacity** (TLC, most constant point in the respiratory cycle) or near TLC if TLC is difficult.
- The patient **exhales <u>immediately</u>**, because breath holding may affect FeNO.

<u>Exhalation phase</u>

Two factors are critical in ensuring reproducible and standardized measurements of lower respiratory tract exhaled NO:

(1) Exclusion of nasal NO
   a. Closure of the velopharyngeal aperture during exhalation by exhaling against an expiratory resistance with subjects asked to maintain a positive mouthpiece pressure (at least 5 cm H_2O and less than 20 cm H_2O)

(2) Standardization of exhalation flow rate.
   a. A **flow rate of 0.05 L/second (BTPS)** appears to be a reasonable compromise between measurement sensitivity and patient comfort, is acceptable to and reproducible in children and adults.
   b. In all cases, the **expiration flow should be clearly recorded** and reported in any publications.

Interpretation of NO Single-Breath Profiles

Constant flow rate exhalations result in a single-breath NO profile (exhaled NO vs. time plot) that consists of a washout phase followed by an NO plateau, which is usually reproducible and flat but may slope up or down.
The washout phase is sometimes followed by an early NO peak before the plateau.
→ Early peaks are ignored, and only NO plateaus are interpreted.

<u>Plateau Definition</u>

- The duration of exhalation must be at least 6 seconds for children >12 years and adults
- The exhaled volume must be at least 0.3 L in adults at an exhalation flow rate of 0.05 L/second to allow the airway compartment to be washed out and a reasonable plateau achieved.

- The plateau concentration in NO should be evaluated over a 3-second (0.15 L) window of the exhalation profile according to the following guidelines:
   - The plateau can be considered to begin at Point A and end at Point B (*see* Figure 1).

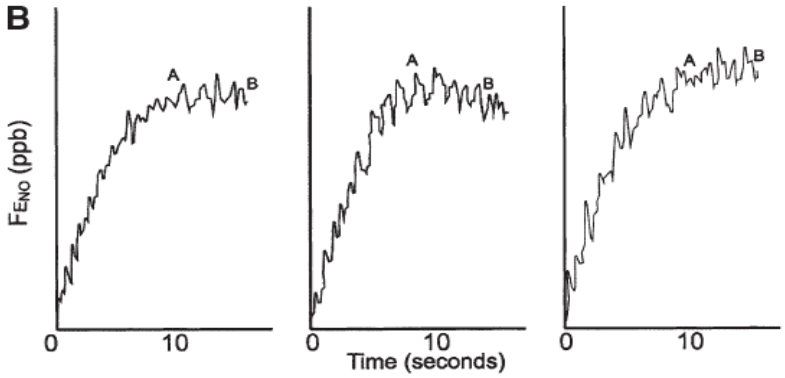


Figure 1. Schematic diagram of exhaled NO and pressure profiles showing horizontal, up- and downsloping NO plateaus with the start (A) and the end (B) of an NO plateau as defined in the text.
(from: ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide (2005). *Am J Respir Crit Care Med* 171: 912–930.)

- The magnitude of the slope should be minimized using the following criteria:
  - Points A and B should be chosen to define the first 3-second window in the exhaled concentration profile such that the absolute magnitude of A–B is less than 10%.
  - No point within the 3-second window should deviate from either the value at Point A or B by more than 10%.
  - The plateau concentration, FeNO, is then defined at the mean concentration over this 3-second window.
  - Once a 3-second plateau is achieved, there is no reason to continue the exhalation.

- For FeNO values of less than 10 ppb, the 10% plateau criterion may be difficult to fulfill because of instrument variability and patient flow rate control variability; in such cases:
  - a change of 1 ppb or less between Points A and B is an acceptable plateau.

Online electronic analysis of NO profiles allows automatic identification of a valid NO plateau according to these criteria. At the recommended flow rate of 0.05 L/second, plateaus are general flat and clearly discernible.

- Repeated, reproducible exhalations should be performed to obtain **at least one NO plateau value**.
- **At least 30 seconds of relaxed tidal breathing off the NO measurement circuit** should elapse **between exhalations to allow subjects to rest**. Care must be taken not to exhaust the patient when repeated exhalations are unsatisfactory.

Appendix

Question to be asked before NO measurement and spirometry

1) Did you smoke today? Yes/No
   - If yes, when did you smoke last? _____hours ago.

Have you been staying at indoor locations where someone else smoked today?
   - If yes, the smoke exposure is how long ago? _____hours ago.

2) Do you have an on-going respiratory infection or cold? Yes/No

3) Have you had asthma symptoms in the last week? Yes/No

4) Did you take medication today? Yes/No
   - If yes, which? (please specify) _____

5) Do you have a runny or blocked nose today? Yes/No

6) Did you eat (meal, snack) or drink today? Yes/No
   - If yes, when did you eat last? _____ hours ago.
   - If yes, when did you drink last? _____ hours ago.

7) Have you in the past 6 hours, had any food with caffeine, (chocolate, cola, ice tea, energy drink,
tea, green tea, coffee). Yes/No
   - If yes, when was your last caffeine intake? _____hours ago
   - And how much? (1) A little bit (small piece of chocolate, small drink)/ (2) a lot (bar of chocolate, bottle or can of drink).

MeDALL

Standard Operating Procedures Spirometry

ERS/ATS guidelines

All cohorts apply the ERS/ATS guidelines for spirometry.
The following descriptions are selected sections from the ERS/ATS guideline:
Series "ATS/ERS Task Force: Standardisatin of Lung Function Testing": Standardisation of spirometry. (2005) *Eur Respir J*; 26:319-338.

Indices to be determined by the MeDALL WP4 cohorts

1) Forced vital capacity (FVC)
2) Forced expiratory volume in one second (FEV_1),
3) FEV6, FEV.50, FEV.75
4) Mean forced expiratory flow between 25% and 75% of the FVC (FEF25-75%)
5) Maximal instantaneous forced expiratory flow where 50 % of the FVC remains to be expired (MEF50)
6) Peak expiratory flow (PEF)

Quality Control

- Temperature and humidity will be recorded in the room where spirometry is measured.
- A log of calibration results is maintained;
- the documentation of repairs or other alterations which return the equipment to acceptable operation;
- the dates of computer software and hardware updates or changes;
- if equipment is changed or relocated (e.g. industrial surveys), calibration checks and quality-control procedures must be repeated before further testing begins.

Calibration

- The syringe used to check the volume calibration of spirometers must have an accuracy of ±15 mL or ±0.5% of the full scale (15 mL for a 3-L syringe).
- The manufacturer must provide recommendations concerning appropriate intervals between syringe calibration checks. Calibration syringes should be periodically (e.g. monthly) leak tested at more than one volume up to their maximum (this can be done by attempting to empty them with the outlet corked).
- A dropped or damaged syringe should be considered out of calibration until it is checked.

- A calibration check is different from calibration and is the procedure used to validate that the device is within calibration limits, e.g. ±3% of true.
- If a device fails its calibration check, a new calibration procedure or equipment maintenance is required.
- Calibration checks must be undertaken daily, or more frequently, if specified by the manufacturer.

Quality control for volume-measuring devices
The volume accuracy of the spirometer must be checked at least daily, with a single discharge of a 3-L calibrated syringe.

More frequent checks may be required:

1) during industrial surveys or other studies in which a large number of subject manoeuvres are carried out, the equipment's calibration should be checked more frequently than daily
2) when the ambient temperature is changing, volume accuracy must be checked more frequently than daily and the BTPS correction factor appropriately updated.

Check over the entire volume range using a calibrated syringe or an equivalent volume standard. The measured volume should be within ±3.5% of the reading or 65 ml, whichever is greater. This limit includes the 0.5% accuracy limit for a 3-L syringe.

Quality control for flow-measuring devices

With regards to volume accuracy, calibration checks must be undertaken at least daily, using a 3-L syringe discharged at least three times to give a range of flows varying between 0.5 and 12 Ls^{-1} (with 3-L injection times of ~6 s and ~0.5 s).
The volume at each flow should meet the accuracy requirement of ±3.5%.
For devices using disposable flow sensors, a new sensor from the supply used for patient tests should be tested each day. For linearity, a volume calibration check should be performed weekly with a 3-L syringe to deliver three relatively constant flows at a low flow, then three at a mid-range flow and finally three at a high flow. The volumes achieved at each of these flows should each meet the accuracy requirement of ±3.5%.

Test procedure

Three distinct phases to the FVC manoeuvre:

1) maximal inspiration;
2) a ‘‘blast’’ of exhalation;
3) continued complete exhalation to the end of test (EOT).

- Check the spirometer calibration
- Wash hands

- Instruct and demonstrate the test to the subject, to include
    - Correct posture with head slightly elevated
    - Inhale rapidly and completely
    - Position of the mouthpiece (open circuit)
    - Exhale with maximal force

- Perform manoeuvre (closed circuit method)
    - Have subject assume the correct posture
    - Attach nose clip, place mouthpiece in mouth and close lips around the mouthpiece
    - Inhale completely and rapidly with a pause of <1 s at total lung capacity (TLC)
    - Exhale maximally until no more air can be expelled while maintaining an upright posture
    - Repeat instructions as necessary, coaching vigorously

- Repeat for a minimum of three manoeuvres in acceptable limits; no more than eight are usually required
- Check test repeatability and perform more manoeuvres as necessary.

Acceptability criteria for manoeuvres

- A satisfactory start of test and a satisfactory end of test (EOT), i.e. a plateau in the volume–time curve.

- The subject performed the manoeuvre with a maximum inspiration, a good start, a smooth continuous exhalation and maximal effort:
    1) without an unsatisfactory start of expiration, characterised by excessive hesitation or false start extrapolated volume or EV .5% of FVC or 0.150 L, whichever is greater;
    2) without coughing during the first second of the manoeuvre, thereby affecting the measured FEV_1 value, or any other cough that interferes with the measurement of accurate results;
    3) without early termination of expiration (see End of test criteria section);
    4) without a Valsalva manoeuvre (glottis closure) or hesitation during the manoeuvre that causes a cessation of airflow, which precludes accurate measurement of FEV_1 or FVC;
    5) without a leak;
    6) without an obstructed mouthpiece (e.g. obstruction due to the tongue being placed in front of the mouthpiece, or teeth in front of the mouthpiece, or mouthpiece deformation due to biting);
    7) without evidence of an extra breath being taken during the manoeuvre.

It should be noted that a *usable curve* must only *meet conditions 1 and 2* above, while an *acceptable curve* must *meet all of the above seven conditions*.

- The reporting format should include qualifiers indicating the acceptability of each manoeuvre.
- Records of failed such manoeuvres should be retained since they may contain useful information.

Between-manoeuvre criteria

- After three acceptable spirograms have been obtained, apply the following tests
    - The two largest values of FVC must be within 0.150 L of each other
    - The two largest values of FEV_1 must be within 0.150 L of each other

- If both of these criteria are met, the test session may be concluded
- If both of these criteria are not met,

    - continue testing until both of the criteria are met with analysis of additional acceptable spirograms or
    - A total of eight tests have been performed (optional) or
    - The patient/subject cannot or should not continue

Save, as a minimum, three satisfactory manoeuvres. In total, eight manoeuvres are generally the upper limit (for an overview of the application of acceptability and repeatability criteria please see Figure 1).

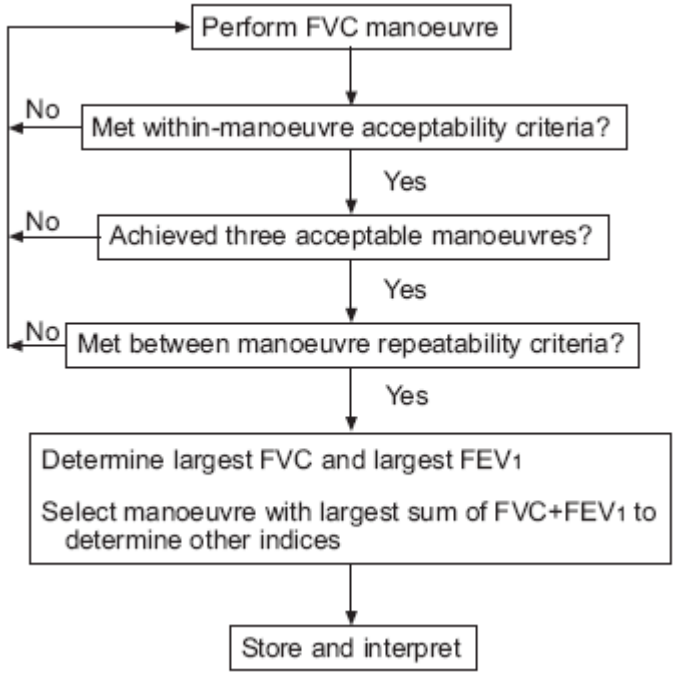


Figure 1. Application of acceptability and repeatability criteria.
(Ref: Series "ATS/ERS Task Force: Standardisatin of Lung Function Testing": Standardisation of spirometry. (2005) *Eur Respir J*; 26:319-338.)

SOP for NDD EasyOne® Spirometer

3. Health Assessment Phase II

3.1 Protocol spirometry

3.1.1 Overview

Spirometry is one of the simplest, most effective tests available for the assessment of lung function. A spirometer measures the amount of air a subject inhales or exhales and the rate at which the air is exhaled. The most common spirometric tests require that the subject exhale with as much force as possible after taking a full, deep breath. The subject's effort is called the forced expiratory manoeuvre.

It is an important aim of this study to ensure that all centres are able to execute a common interview and examination schedule and in particular undertake uniform measures for lung function. We will therefore employ the same type of spirometer across all centres - the NDD EasyOne® Spirometer . This is a highly portable spirometer that measures flow and volume by ultra-sound transit time, is endorsed by the ERS and complies with ATS spirometry standards.

Every spirometry session must be performed according to the SOP by study staff or technicians who have undergone the study training. To ensure data integrity equipment must be regularly cleaned and the calibration checked daily according to manufacturers instructions.

3.1.2 Measures

During each session the following measures should be collected:

Forced Vital Capacity (FVC)	The total volume of air exhaled in a forced expiratory manoeuvre. The FVC is useful for detecting restrictive diseases, since lower than expected results may be a sign that the lungs cannot inflate normally. FVC is reduced in people with obstructive and restrictive disorders.
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 and is reduced in individuals with airflow obstruction.
The ratio of FEV_1 to the FVC (FEV_1/FVC)	The most sensitive and specific index of airways obstruction measured by a spirometer. 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. Increasing interest is being shown in the FEV_6, and more particularly in the FEV_1/FEV_6 ratio, as an alternative to the FEV_1/FVC ratio.
The ratio of FEV_1 to the FEV_6 (FEV_1/FEV_6)	An alternative to the FEV_1/FVC ratio.
FEF 25-75%	
MEF 50	Mid Expiratory Flow at 50% of the Vital Capacity
PEF	Peak Expiratory Flow

3.1.3 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 in a quiet area of the school building. These conditions will improve the quality and reproducibility of the results. For safety, the participant should be seated in a chair with no 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
- Questionnaires

3.1.5 Calibration

The EasyOne Spirometer has been designed to need no calibration. However, a calibration check should be carried out daily to ensure that the spirometer is reading accurately. 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. If spirometry is done in the field (outside a clinical setting), it is preferable to keep the spirometer and calibration syringe together overnight to avoid temperature differences at the time of calibration.

3.1.6 Medication use prior to testing

In order to provide a valid lung function assessment, volunteers should be asked about the use of bronchodilators in the last 4 hours. If the participant has used a beta-2-agonist inhaler or an anit-muscarinic inhaler in the last four hours, consider waiting until four hours since last use has elapsed. If this is not possible, proceed.

If the volunteer has not been able to comply with these waiting periods, the spirometry should be done anyway (unless it is feasible that the participant be tested on another day) and medication usage in the last 24 hours should be recorded.

3.1.7 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 volunteer. These situations are at the discretion of the staff member but may include acute back pain, a respiratory tract infection with unresolved symptoms in the week prior to the visit, or very 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 volunteers 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.

3.1.8 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
- they are taking medication for tuberculosis
- 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 volunteer's safety

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

3.1.9 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. A copy of this document should be kept with each spirometer in case questions about the use of the spirometer arise during testing.

Volunteer information should be entered into the spirometer as prompted. In the ID field enter the subject's unique ID. Do not enter the centre number or sample number. Do not enter the subject's name.

As prompted enter the age, height, weight, ethnic category, gender, smoking status and the ID of the lung function assistant undertaking the test.

If after safety questions it is decided to reschedule the session, document the potential safety issues in questionnaire II. Ensure that the same questionnaire is recalled for use if a second visit is arranged.

If testing is to proceed offer volunteers 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 volunteer 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 should wash their hands before the start of the test and use a tissue to remove mouthpieces (the Spirette) from the storage container. If appropriate allow the volunteer 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.

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.

3.1.9.1 The manoeuvre

Explain that the volunteer should take in as deep a breath as possible, and when his/her lungs are totally full, quickly position the mouthpiece and BLAST out the air as hard and as fast as possible. A vigorous demonstration of the manoeuvre will help the volunteer understand the manoeuvre much more quickly. Demonstrate the correct positioning of the mouthpiece. Take a deep breath and emphasize the full depth of inhalation. Then demonstrate a dramatic blast out as fast as possible. Because the adequacy of these manoeuvres is highly dependent on volunteer effort, staff must guide the volunteer through the technique. It is extremely important to inhale as fully as possible and to exhale very forcefully, and as much as possible. Tell the volunteer when to start taking in a deep breath and to put the mouthpiece in his/her mouth. Then tell them to blast out the air and to continue exhaling for at least 6 seconds. Observe their body language as he/she attempts to follow the instructions, and encourage them to continue blowing out smoothly without re-breathing. Instruct the participant to remain erect and not to bend over during the manoeuvre.

Follow the procedures outlined in sections 5.2 to 5.4 of the NDD EasyGuide users' manual. Follow the computer prompts until a successful test session has been obtained. A successful test session is defined as at least three acceptable manoeuvres, with both the two best FEV_1s and the two best FVCs from these manoeuvres within 200 ml of each other.

3.1.9.1 Acceptable and reproducible manoeuvres

"Acceptable" is defined as a manoeuvre that is free from error. "Reproducible" is defined as being without excessive variability between manoeuvres.
Many factors will result in error, including hesitation or false starts, cough, variable effort, glottis closure, early termination and leaks.
Three acceptable manoeuvres are needed to be 'reproducible'. The two highest values for FVC and FEV_1 taken from acceptable forced expiratory manoeuvres should not vary more than 200 millilitres from the second highest FVC and FEV_1. It is also important to monitor the volume-time curves to determine if the size and shapes of the curves are reproducible.

When errors occur, review them with the volunteer 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 volunteer tries again and the reproducibility criteria are not met, continue the test as needed (up to a total of five manoeuvres), assuming that the volunteer is able to continue.

Some volunteers 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. Previous studies have shown that inability to perform reproducible spirometry, even with good coaching, is an important risk factor in predicting future health.

Centres may wish to discuss lung function results with their volunteers and will therefore wish to compare test results with predicted values. The NDD EasyOne spirometer offers a number of published predicted values, most of which were derived from studies of largely Caucasian participants. We recommend the use of the ERS/ECC's reference values.

The quality grades allow you to assess the reliability of the measurement result. Quality grades A to C indicate a reliable result. A quality grade between D and F indicates

inadequate test quality. The result must then be interpreted with caution. The quality ratings can be activated or deactivated under “Configuration”. See also Chapter 8.

The table below defines the criteria for the classification of quality grades:

Rating	Criteria in Diagnostic mode	Criteria in Frontline and NLHEP mode
A	At least 3 acceptable tests (for age < 6: 2 acceptable) AND the difference between the best two FEV1 and FVC values is equal to or less than 100ml (80ml if FVC < 1.0 L; for age < 6: 80ml or 8% of FVC whichever is greater)	At least 2 acceptable tests AND the difference between the two FEV1 and FEV6 values is equal to or less than 100ml
B	At least 3 acceptable tests (for age < 6: 2 acceptable) AND the difference between the best two FEV1 and FVC values is equal to or less than 150ml (100ml if FVC < 1.0 L; for age < 6: 100ml or 10% of FVC whichever is greater)	At least 2 acceptable tests AND the difference between the two FEV1 and FEV6 values is equal to or less than 150 ml
C	At least 2 acceptable tests AND the difference between the best two FEV1 and FVC values is equal to or less than 200ml (150ml if FVC < 1.0 L; for age < 6: 150ml or 15% of FVC whichever is greater)	At least 2 acceptable tests AND the difference between the two FEV1 and FEV6 values is equal to or less than 200 ml
D	At least 2 acceptable trials but the results are not reproducible. Quality message "Result not reproducible" OR only one acceptable trial. Quality message: “Only one acceptable trial”	At least 2 acceptable trials but the results are not reproducible. Quality message "Result not reproducible" OR only one acceptable test. Quality message "Only one acceptable trial”
D	No acceptable test available	No acceptable test available

3.1.10 Reference values

Centres may wish to discuss lung function results with their volunteers and will therefore wish to compare test results with predicted values. The NDD EasyOne spirometer offers a number of published predicted values, most of which were derived from studies of largely Caucasian participants. Four ethnic correction settings are available that allow you to customize the amount of adjustment that is made for selected racial groupings. Consult the EasyGuide users’ manual, sections 8 and 12, for more information regarding the use of prediction equations.

Test settings:

Related to	Choice
Predicted	ERS/ECC
Best Value Selection “ValueSel”	Best Value
Interpretation	NONE

3.1.11 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 volunteer. Nose clips should be thoroughly cleaned after each use with hot water and detergent, allowed to dry and then wiped with alcohol.

It is recommended that staff and, if possible the volunteers, wash their hands before and after testing and that proper attention be given to environmental controls in settings where tuberculosis or other diseases spread by droplet nuclei are likely occur. Volunteers with evidence of obvious upper respiratory infections should not be tested, but rather asked if they may be tested at a later date.

On each day it will be used, the spirometer should be first calibrated with a 3.00-Liter syringe that has been stored next to the spirometer. The NDD calibration adapter for connecting the syringe with the spirometer is required for calibration. Beyond battery replacement and the calibration check, no maintenance is required or recommended on the spirometer or cradle. No service should be performed on the spirometer except by manufacturer-authorised personnel.

3.1.12 Data Transfer

NDD EasyWare PC-software will be used, which is compatible with a PC running Microsoft Windows 98/ME/2000/XP. EasyWare software is available in English, French, Spanish, German and Italian. Ideally data should be transferred to a local PC daily and stored in mbd format (Access). A pulmonologist of your hospital should check the data of each spirometry to ensure that the curves are nice and that spirometries are performed adequately.

BELOW IS ONE QUESTIONNAIRE BASED ON THE PUPIL'S AND TEACHER'S QUESTIONNAIRES AND COMMENTS

Lung function questionnaire (child) :

Fieldworker: _____

Date: ☐☐ ☐☐ 2 0 ☐☐

(day) (month) (year)

Child's name: _____ ID: _____

Child's Height ☐ ☐☐ (metres)

Child's Weight ☐☐☐ (kilograms)

Child's Date of Birth

☐☐ ☐☐ 2 0 0 ☐

(day) (month) (year)

Child's Sex
- ☐ Male
- ☐ Female

Time of Day (24 ☐☐ : ☐☐ hrs)

(Hours) (Minutes)

When making an appointment make sure that parents understand that children taking medicines on a regular basis, should not take medicines for at least 4-8h prior to the lung function test. Before starting the questionnaire please ask the following question:

Have you used an inhaler (puffer) in the last hour?
- ☐ No
- ☐ Yes

If 'Yes' to this questions, delay lung function tests until one hour after the last inhaler use. Delay lung function tests one hour if children have used short-acting inhalers and until 4 hours if children have used long-acting inhalers such as beta2-agonists and anti-muscarinics.

1. Do you have a (nose) cold at this moment?
   - ☐ No
   - ☐ Yes

2. Have you had an attack of asthma in the last 3 days?
   - ☐ No
   - ☐ Yes

3. Have you taken any medication for asthma in the last 24 hours (including inhalers, aerosols or tablets)?

☐ No
☐ Yes

END

Could use additional questions from the teacher's questionnaire below??

3. Have you had a respiratory infection in the last 3 weeks?

If 'No' go to question 4. If 'Yes' proceed with question 3.1

3.1 How many days ago did it end?

☐☐ (days)

4. Have you used an inhaler in the last 24 hours?

☐ No
☐ Yes

If 'No' go to question 5; if 'Yes' proceed with question 4.1:

4.1 Have you used a beta-2-agonist inhaler or an anti-muscarinic inhaler an inhaler in the last 4 hours?

☐ No
☐ Yes

If 'yes' to question 4.1 (participant has used a beta-2-agonist inhaler or an anti-muscarinic inhaler in the last four hours), consider waiting until four hours since last use has elapsed. If this is not possible, proceed.

5. Have you used any other medicine (including pills, capsules or suppositories) to help your breathing, or any oral anti-muscarinic in the last 24 hours?

☐ No
☐ Yes

If 'No' end of questionnaire; if 'Yes' proceed with question 5.1:

5.1 What medicine(s) did you take and how many hours ago did you take it?

_____

_____

_____

END

MeDALL

Standard Operating Procedures Parental Stress (SDQ)

- **PSI-SF and ADHD scale is optional**

MeDALL

Standard Operating Procedures

DNA preparation for methylation study (WP6)

Genome Analysis Facility
Dept Genetics UMC Groningen

Introduction

To study the genome wide DNA-methylation status in cases of allergic disease and controls the Illumina Infinium methylation arrays are used. The measurements will be carried out in the high throughput laboratory of the Genome Analysis Facility of the Department of Genetics in Groningen. To ensure a successful and rapid processing of the DNA-samples the DNA-quality needs to meet a few criteria and there are special demands about the format of the sample delivery.

Al the required information for the preparation of the DNA-samples is described in this document.

DNA-collection

Draw blood into 3 ml tubes containing anticoagulant (EDTA). Be sure to draw the full volume to ensure the correct blood-to-anticoagulant ratio. The minimum amount needed depends on the method applied to isolate DNA. For the recommended method at least 1 ml of blood is needed. Invert the tubes carefully 10 times to mix the blood and anticoagulant and store at room temperature. The use of 'fresh' blood is preferred for DNA isolation, but do not store the blood longer than 5 days. If processing is not possible within 5 days, store the blood in a freezer (-20 ℃) after collection.

DNA-isolation

To isolate DNA out of multiple blood samples the *Qiagen QiaAmp DNA 96 blood kit* is recommended. Using this kit it is possible to isolate 192 DNA-samples in one day, starting from 1 ml blood samples. However, an additional precipitation of the DNA with iso-propanol, glycoblue (Ambion) and sodiumacetate is needed to obtain a more concentrated DNA-solution. In case of problems please mail to p.van.der.vlies@medgen.umcg.nl
Alternatively many other DNA-isolation methods could be used. The quality of DNA isolated by most isolation-method meets usually the criteria for a successful Infinium assay.

DNA-quality

The quality of the isolated DNA should be verified with a spectrophotometer, ideally by the Nanodrop spectrophotometer.
The quality criteria are:

- Concentration should be between 50 ng/µl and 100 ng/µl.
- The OD 260/280 ratio should be between 1.8 and 2.0
- The OD 260/230 ratio should be above 1.5
- The DNA should by high molecular, fragments of > 5kb. (This could by checked using agarose gelelectroforesis of a subset of the samples)

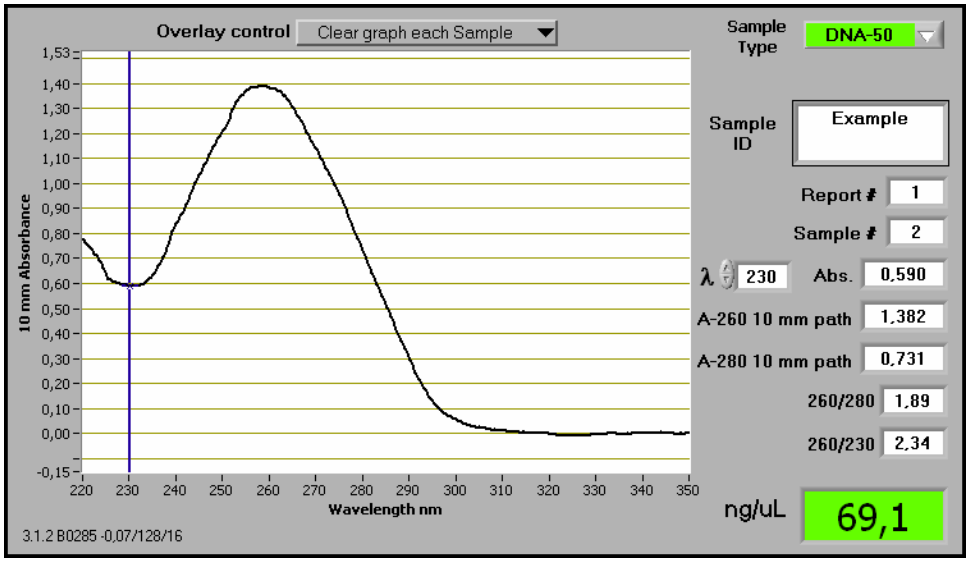


Figure: Example of a nanodrop measurement of a good quality DNA-sample.

Format

- Exactly 30 µl of the DNA-solution (50 ng/µl-100 ng/µl) should be transferred to 96-well plates (Greiner Bio-One, PCR-plate full skirted, order nr. 652270).
- Add samples in the well-order A1, B1, C1, D1 etc, don't leave any well blank between samples. Administrate the sample ID's in a special XLS-file according to the guidelines below. A unique plate-name should be given to each 96-well-plate, use a permanent marker to annotate each plate on two sides.
- The 96-well plates should be firmly closed with a Thermo-seal (*Thermo scientific order nr. AB-0559*) using a Heat plate sealer. Alternatively, if a heat plate sealer is not available, adhesive PCR Foil seals (*Thermo scientific order nr. AB-0626*) could be used exclusively.
- After the DNA-solution is transferred to a 96 well-plate and the plate is sealed properly, the plates should be stored at -20°C.

XLS-file

nr	centre	plate	well	sample ID	concentration (ng/ul)	gender
1	UMCG	UMCG-1	A1	470	66	m
2	UMCG	UMCG-1	B1	802	56	f
3	UMCG	UMCG-1	C1	655	89	f
4	UMCG	UMCG-1	D1	463	78	m
5	UMCG	UMCG-1	E1	228	91	f
6	UMCG	UMCG-1	F1	109	67	f
7	UMCG	UMCG-1	G1	467	89	m
8	UMCG	UMCG-1	H1	928	67	m
9	UMCG	UMCG-1	A2	150	55	f
10	UMCG	UMCG-1	B2		98	

Figure: Example of the attached XLS-file

All sample-IDs and corresponding wells in the 96-well plate should be administrated in a XLS-file. (See figure). In this XLS file all needed information is summarized. For quality reasons the gender should also be administrated.

Posting

The 96-well plates containing the frozen samples, which are selected and ordered by WP6 (Dirkje Postma, Gerard Koppelman) should be transported by a courier-company to the laboratory of the Genome Analysis Facility of the department Genetics of the UMCG. The shipment should be carried out in boxes containing a sufficient amount of dry-ice.

To avoid any delay during the weekend, please ship the material in the beginning of the week.

The address is:

Genome Analysis Facility, MeDALL research
Dept. Genetics UMCG
Hanzeplein 1, Ingang 47 Oostersingel
Room E2.030
9713GZ Groningen
The Netherlands

Please email the XLS-file with the sample-information to: p.van.der.vlies@medgen.umcg.nl
(This email address could also be used for remaining questions.)

MeDALL

Standard Operating Procedures

Blood collection and Serum samples for biomarker analyses (WP6) and determination of allergen-specific IgE and other antibody classes and subclasses (WP5)

Serum samples for biomarker analyses and determination of allergen-specific IgE and other antibody classes and subclasses - Collection, Packaging and Transport Protocol

Purpose

In WP5, serum samples will be analysed by protein-microarray for antibody-reactivity to approximately 150 allergen-molecules and to a panel of bacterial toxins that are contained in the array. Reactivity of different antibody isotypes can be distinguished.
In WP6, a panel of biomarkers will be measured in serum from selected blood samples drawn in MeDALL.

Materials:

Blood collection:

- Labelled 8.5 ml serum tubes with separating gel (red cap - e.g.: Becton Dickinson Vacutainer SSTAdvancetube) for centrifuge.
- Blood sampling kit: needles (19G; butterfly), tube rack, cuff, rubber gloves, disinfectant, gauze bandages, medical tape, disposal box for needles. Different blood sampling systems (e.g. Vacutainer, Kabe, Sarstedt) can be used.

Serum storage:

- Polypropylene cryotubes with internal thread (1.5 ml – e.g. Nunc), for preservation of serum.
- Boxes (up to 81 tubes e.g.: B50 GLW company).

Procedure for blood collection:

- Blood should be collected only by trained personnel using aseptic methods.
- The extraction of blood must be made with the highest measures of sterility.
- Sampling location should be an isolated, peaceful area (e.g., a separate room) with all the necessary equipment prepared beforehand.
- Date and time of blood sampling should be noted on a lab-doc-sheet. Also, whether participants were required to fast should be specified.

Procedures for serum extraction:

- After blood has been collected, tubes should be inverted gently 5 to 6 times.
- Blood should be left to clot for a period of not more than 4 hours (BD recommended 2h) at room temperature. It is important to avoid exceeding these times because of excessive hemolysis. When vacuum gel tubes are used, the temperature should be at least 20℃ (optimum 20-22° C) because the gel viscosity changes in colder temperature.
- Samples should be centrifuged: 1000-1300 RCF (g) for 10-15 minutes.
- The serum should be promptly separated from clot or cells. For WP6 two aliquots of 500 μl of serum each should be transferred to clean 1.5 ml cryotubes (each aliquot of 500 μl serum should be transferred to one clean 1.5 ml cryotube). For WP5 one aliquot of 500 μl of serum each should be transferred to clean 1.5 ml cryotubes. Collection and storage of a fourth 500 μl aliquot is also recommended.
- The white cell layer should not be transferred. Vacuum gel tube separated serum can be poured to a clean tube.
- Cryotubes should be labelled ahead of time with centre and subject's numeric ID. If possible, barcodes should be included as well. Do not include any personal identifiers other than numeric IDs on the specimens and specimen containers.
- Cryotubes will be placed sequentially in the boxes and their location annotated electronically.
- All serum aliquots must be organized in electronic inventories and stored at -80ºC by individual studies until shipping of selected aliquots to CREAL for biomarker analysis.

Transport of samples:

- Serum samples / IDs that are selected for biomarker analysis will be communicated promptly by CREAL to individual studies.
- These serum aliquots need to be placed in new B50 GLW boxes for shipping.
- For transport, samples should be properly packed and each of them clearly marked with original labels. If multiple boxes are shipped, each of them should be clearly marked with sequential numbers.
- Each shipment should be accompanied by a specific submission form including shipping lists with all IDs that have been packed into the transport boxes and their locations in the boxes. Samples must be transported on dry ice and should be delivered overnight.

- Label the transport container appropriately, using permanent marker. Forms required by the specific courier and by specific regulations should also be filled.
- Samples should be shipped preferentially during the first days of the week.
- Before planning any shipping, for WP6 (Biomarkers) please contact Iris Lavi at CREAL (contact info below) to coordinate shipping and receipt of the samples.

For biomarker analyses, samples which are **selected by CREAL and ordered by WP6** (Iris Lavi, Stefano Guerra) should be shipped to (for each selected child, **2 aliquots of 0.5ml**):

Dr Iris Lavi (WP6)
Centre de Recerca en Epidemiologia Ambiental (CREAL)
Doctor Aiguader, 88
E-08003 Barcelona, Spain
E-mail: ilavi@creal.cat
Telephone: +34-93-2147355

For protein-microarray analyses, samples which are **selected and ordered by WP5** (Christian Lupinek, Rudolf Valenta) should be shipped to (for each selected child, **1 aliquot of 0.5ml**):

Prof. Rudolf Valenta (WP5)
Christian Doppler Laboratory for Allergy Research
Division of Immunopathology
Dep. Of Pathophysiology and Allergy Research
Center for Pathophysiology, Infectiology and Immunology
Medical University of Vienna
Währinger Gürtel 18-20, 3Q
A-1090 Vienna, Austria
E-mail: rudolf.valenta@meduniwien.ac.at
Telephone: +43-1404005108/ +43-1404005109
Fax: +43-1404005130

MeDALL Serum Submission Form for Biomarker Analyses

To be completed at CREAL
Received by: _____
Date received: _____
Box Numbers: _____
Comments:
_____
_____
_____
_____
_____

Ship date: _____ Shipped by:
Centre/study:
_____
Address:
_____
Contact person:
_____
Tel 1:
_____
Tel 2:_____
E-mail:
_____

Sample information:
Type: **Serum samples**
# of samples: _____
Comments:
_____
_____
_____
_____
_____
_____

Ship to:

Iris Lavi· CREAL ·
Doctor Aiguader, 88 ·
E-08003 Barcelona · Spain
Tel +34 93 214 73 55 ·
e-mail: ilavi@creal.cat

MedALL Serum Submission Form for Biomarker Analyses

Sample IDs

Please type or write sample IDs in the space provided below.
- OR - You may attach other documentation listing sample IDs to this form.

Comments / Special instructions

_____
_____
_____
_____
_____