The European Community Respiratory Health Survey

MAIN PROTOCOL

Background

Since 1990 the European Community's Concerted Action on Asthma Prevalence and Risk Factors (the European Community Respiratory Health Survey) has been collecting information on the variation in asthma prevalence, known or suspected risk factors for atopy and asthma, and information on the management of asthma. The study has involved 33 centres in 11 countries in the European Community, seven centres in five COST (Co-operation in Science and Technical Research) states in Europe, and 16 centres in eight other countries who are participating at their own expense.

Asthma is a serious cause of morbidity in the European Community

Asthma is an increasingly serious cause of morbidity in many countries. When this programme of research began, mortality rates from asthma had been increasing since the mid-1970s and were a serious source of concern in several countries, including England and Wales, France, Germany, Denmark, the United States, and the Scandinavian countries (Bredkjaer, personal communication). The changes in mortality were particularly notable as mortality from causes of death that were thought to be amenable to medical intervention had been falling rapidly since the early 1950s in almost all countries. Though these increases in mortality now show signs of falling they have been accompanied by even greater increases in discharge rates from hospitals, particularly among children. Again, this is a trend that has been noted in several countries including England and Wales, the United States and New Zealand, and is not a local phenomenon. In England and Wales there was also a marked increase in consultations with general practitioners for asthma and hay fever between the early 1970s and the early 1980s. Although these changes might have been explained by alterations in medical practice and in the criteria used to diagnose asthma, such an explanation seems unlikely to account for such widespread changes.

The hypothesis that these common trends were due at least in part to an increase in asthma prevalence now looks increasingly likely. Analysis of trends in mortality and admissions for asthma both show 'cohort effects' suggesting that the increase in both has been, at least in part, due to changes between generations rather than simple changes over time that affect all generations equally and simultaneously.
This is at least compatible with the hypothesis that the incidence of disease has changed between the generations. More direct evidence comes from surveys of asthma. These have been criticised in the past either because they have relied on a diagnosis of asthma, a diagnostic term that may have become more fashionable with time, or because they have been based on local surveys where the population may have changed between surveys. There is now more direct evidence that does not rely on diagnostic terms. Surveys that have documented increases in the prevalence of asthma-like symptoms over time in identifiable populations include studies from the United Kingdom, Australia, New Zealand and the United States. Though these studies could, individually, be due to migration or other changes in the local population over time, these are unlikely to explain such consistent results, and in England the National Study of Health and Growth has shown an increase in the prevalence of 'persistent wheeze' in a representative sample of English school children since the start of the study in the early 1970s. This study is being spread over a number of different locations and is unlikely to be influenced strongly by local effects of migration.

Asthma is a potentially preventable condition

These findings imply that asthma is a potentially preventable disease. The evidence for this comes from the wide variability of asthma prevalence in populations that are unlikely to have important genetic differences. This is most marked in the developing countries where very large increases in asthma prevalence have been noted in the urbanised or more westernised areas. Again, this trend has been noted in several different areas of the world, including southern Africa, west Africa, Papua New Guinea and the Pacific Islands. These changes are far too rapid and large to be interpreted as genetic changes and must be related to some environmental risk. Similar, though less dramatic variation in prevalence has also been noted in England, where the annual prevalence of nocturnal dyspnoea, a symptom of moderately severe asthma, varied in men aged 20 to 44 years from 2.8% to 4.8% in different local authority districts sampled in 1986, a significantly greater variation than could be expected by chance.

The reasons for these variations are, however, unknown. They could in part be due to differences in the prevalence of atopy, a known risk factor for asthma. The Tokelau migrants, when living in New Zealand, showed an increase in the prevalence of rhinitis and eczema as well as asthma, and this supports this view. However, Godfrey failed to show a comparable difference in skin sensitivity.
between urban and rural Gambians to explain the difference he found in the prevalence of asthma. Even if there are wide variations in the prevalence of atopy there may be other reasons for differences in asthma prevalence.

If the prevalence of atopy does vary it could be partly due to variation in genetic susceptibility, but it is likely to be more than just this. There is reason to believe that the prevalence of atopy has been rising as well as the prevalence of asthma. This evidence is based in part on evidence that clinically defined conditions, such as rhinitis and eczema, have also been increasing in prevalence\textsuperscript{12,13,25,26} and partly on less abundant evidence that the prevalence of atopy as measured by skin tests\textsuperscript{27} or specific IgE\textsuperscript{28} might be rising. This rapid increase implies an environmental cause. Exposure to allergens might be expected to have an effect on the prevalence of allergic symptoms but the evidence for increasing asthma prevalence seems out of all proportion to any increase in exposure to common allergens. One alternative suggestion might be that maternal smoking during pregnancy has increased susceptibility to sensitisation with allergens in early life and that this partly accounts for the increase in asthma prevalence. Though not all studies have agreed with these findings, maternal smoking has been associated with a high cord IgE, a higher incidence of atopic conditions in the first year of life\textsuperscript{29} and a higher prevalence of asthma in the early teens.\textsuperscript{30} The increase in mortality from asthma has also occurred in England and Wales in generations whose mothers smoked increasing amounts of cigarettes.

As already stated, variations in atopic response are unlikely to account for all the variation in asthma prevalence. Another group of environmental risks that have recently received a great deal of attention are those that cause inflammation of the airway. These include respiratory infections, air pollutants and some occupational exposures. There is substantial evidence that all of these agents can and do incite bronchoconstriction in asthmatics. They would also, for that reason, be expected to increase the prevalence of symptomatic asthma. Whether they can induce asthma in previously normal subjects other than through some allergic mechanism is more open to dispute. Nevertheless, they are potential risks for asthma and some account needs to be taken of them in a comprehensive assessment of risk factors for asthma in the community.

A further risk factor that has only been recognised recently is that of dietary sodium. This was initially linked to the geographical distribution of asthma mortality in England and Wales.\textsuperscript{31} Although this relationship was an ecological association based
on crude estimates of regional sodium consumption it has stood up well to subsequent investigation. The initial study showed significant associations between table salt purchases in different regions of England and Wales and asthma mortality in adult men and children of both sexes, but not in adult women. A subsequent survey of men living in two villages in Hampshire showed that the bronchial response to histamine was significantly associated with sodium excretion after adjusting for possible confounders, such as body size, smoking and skin sensitivity to common allergens. Finally, two trials have shown that altering the dietary sodium will affect the response to histamine in men, but not in women.

Reported asthma mortality rates vary markedly across Europe, as do treatment patterns. Prevalence rates vary significantly between different areas of England. It is unknown whether this is true for Europe as a whole. Though a number of groups have completed prevalence studies, the methods used have not been consistent between countries.

**Justification for co-ordination**

The European Community Respiratory Health Survey (ECRHS) was designed to cover all areas of the European Community and has included other areas also. There were two reasons for such a design. The first was that the environmental and cultural variation across Europe was likely to be far greater than that across any individual area, region or country. The second was that the cost of such a study would have been prohibitive in any single country.
Specific objectives of the study

1. To estimate the variation in the prevalence of asthma, asthma-like symptoms and bronchial lability in Europe.

2. To estimate variation in exposure to known or suspected risk factors for asthma; to measure their association with asthma and to further assess the extent to which they explain variations in prevalence across Europe.

3. To estimate the variation in treatment practice for asthma in the European Community.

Study design

THE SAMPLE

Selection of areas

It would not have been feasible to select a random sample of areas to study from each country. However, the selection of highly unrepresentative samples is less likely if large areas are selected and if the populations / areas to be studied are defined by pre-existing administrative boundaries. For this reason the following guide-lines were given for the selection of areas for this study:

1. Areas should be selected by pre-existing administrative boundaries.

2. Areas should have total populations of around 150,000 people.

3. Areas should have up to date sampling frames that could be used to sample 20-44 year old adults.

Number of areas

At least 30 areas throughout the European Community were required in order to allow for some ecological analysis of the differences between areas. The project aimed to collect data from at least three areas in each participating country or region to reduce the confounding effects of countries and languages. At least some ‘within
country' analysis would then be possible to take separate account of the 'between
country' differences.

Selection of subjects

a) Selection of subjects for Stage I (Screening Questionnaire)

Subjects should be a representative sample of 20-44 year old men and women
resident in the areas. The selection of these subjects was ideally made by random
selection from a suitable sampling frame, but it was recognised that this had to be
adapted to local conditions. Individuals who returned a Screening Questionnaire
were called 'responders'. Each centre decided the strategy most likely to maximise
response. The point at which any individual was defined as a 'non-responder', for
example, if an individual had not returned a third questionnaire after 40 days, was
also defined locally. The reason for non-response was determined and coded, but if
it was not possible to obtain information on all non-responders, a random sample of
these was investigated. It was suggested that the initial sample size should take
account of the likely non-response rate in each area with the aim of obtaining 3000
responders, 1500 of each sex.

b) Selection of subjects for Stage II (Main Questionnaire and further tests)

The aim of sampling for the second stage was to provide:

i) a random sample of subjects to be studied,
ii) an additional sample of cases to be selected on symptoms.

The random sample was selected from all individuals who had been included in
Stage I. This sample inevitably included non-responders to Stage I. These subjects
were not approached for Stage II unless, and until, they had become responders to
Stage I, so that the reasons for the non-response to Stage I could be ascertained. The
aim was to obtain 300 of each gender, that is a 20% sample. If response rates to the
two stages were expected to differ then Stage II could be separately over-sampled to
take account of this. The reasons for non-response to Stage II were then determined.

The additional sample consisted of 100-150 symptomatic individuals in each area.
These were selected from Stage I responders who answered 'yes' to any one of
Questions 3, 5 or 6 in the Screening Questionnaire and were not already in the
random sample for Stage II. If there were too many of these a random selection was made. If there were less, then all were eligible for Stage II.

Number of subjects per area

The sample size for the study was set at a minimum of 1,500 of each sex to be administered the Screening Questionnaire in each area, and a minimum of 300 randomly selected subjects of each sex to be administered each of the subsidiary tests, including the Main Questionnaire. Each of the two samples (men and women) were randomly selected from the sampling frame. These sample sizes were selected in order to have a 90% chance of detecting a two-fold variation in the prevalence between any two areas. These sample sizes assume that the prevalence of symptoms is approximately 5% and that the prevalence of hyperresponsiveness is approximately 14%. Estimates of differences in variation in atopy are likely to be more sensitive than this.

THE INSTRUMENTS

The Questionnaires

The questionnaires were developed where possible from pre-existing questionnaires, which had already been used in multinational studies. The questionnaires were tested for comprehensibility and translated, with back translation into English. The Screening Questionnaire was generally sent by post and self-administered, though some centres found that this was not practical. The Main Questionnaire, and the Screening Questionnaire where this could not be self-administered, was administered by trained interviewers.

a) Symptoms and medical history

These questions were taken from the bronchial symptoms questions of the International Union Against Tuberculosis and Lung Disease (IUATLD) questionnaire. A version of this questionnaire had already been used in an extensive survey of symptoms in England and a preliminary study comparing the characteristics of the questionnaire in four European countries had been completed.
b) Occupation and social status

These questions were taken from the Office of Population Census and Surveys' (OPCS) questions on occupation and social status. They are compatible with the European Community Socio-Economic Status Groups.

c) Smoking

Questions on smoking habit were adapted from the American Thoracic Society (ATS) Questionnaire.

d) Home environment

The questions on housing conditions were based on those used in the Children's Health Study (Harvard School of Public Health and Canadian Health and Welfare), which surveyed 24 communities in the US and Canada.

e) Questions on medication and use of services

There was no currently available questionnaire suitable for all countries of the European Community. A new questionnaire was therefore devised.

Allergy tests: skin sensitivity and serum IgE

Atopy is a predisposition to develop an IgE-mediated immune response to environmental allergens that do not sensitize non-atopic individuals. The expression of an atopic phenotype requires the interaction of a partly genetic predisposition with environmental allergen exposure. There are several climatic regions in Europe and each has a different distribution of allergens. Therefore, a broad selection of allergens was used in this study.

a) Skin prick testing

Studies of skin sensitivity are the most practical in epidemiological surveys and are generally acceptable to the public. They give a semi-quantitative measure of sensitisation and are relatively cheap. Skin testing was carried out using Phazets (Pharmacia Diagnostics AB, Uppsala, Sweden), which are lancets pre-coated with standardised lyophilised allergen extracts. Except where local regulations made this
impossible, a standard list of allergens was used in all centres in the survey. Because there are cross-reactions between allergens, and sensitivity to regional allergens may be found outside the region of that allergen's usual distribution, this list included some allergens which are regionally restricted. The allergens that were selected for use in all areas included: *Dermatophagoides pteronyssinus*, cat, *Alternaria alternata*, *Cladosporium herbarum*, timothy grass, birch, *Parietaria judaica*, olive and ragweed, with a positive control (histamine) *Phazet* and a negative control (un-coated) *Phazet*. Each area could add up to two additional allergens of local importance.

b) Serum IgE

Specific and total IgE was measured using the Pharmacia CAP System. Specific IgE was measured against *D pteronyssinus*, grass, cat, *Cladosporium* and a local allergen. These local allergens were birch for northern Europe, *Parietaria* for southern Europe and ragweed for the US and Canada. Although total IgE has poor sensitivity and specificity for clinical atopy, Burrows *et al* report that this is the single best predictor of 'asthma'.

Measurement of bronchial responsiveness (methacholine challenge)

As a consequence of the difficulties in interpreting the relative prevalence of symptoms elicited in different cultures and in different languages, it is necessary to have a more objective measure related to asthma. Although it is recognised that clinical asthma and bronchial responsiveness are not identical, bronchial reactivity has been shown to be a consistent feature of most asthmatics. Bronchial challenge with inhaled agents, such as histamine and methacholine, has been used extensively in epidemiological surveys. These tests have been widely conducted, particularly in Europe, and there are extensively published data available with which to compare results.

The principal objective of the challenge testing was to obtain standardised measurements between areas. The protocol was therefore designed to maximise the comparability of methods between areas and it was most important that areas were able to use the same equipment for performing lung function tests. The following were selected for the standard methodology:

a) Dosimeter
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*Mefar MB3* Dosimeters (Mefar srl, Bovezzi, Italy) was used for the administration of methacholine.

b) Spirometer

*Biomedin* Spirometers (Biomedin srl, Padova, Italy) meet the European Commission standards and have computerised operating systems to ensure quality control to the same standard in each area.

c) Methacholine

Standard methacholine solutions were made up from lyophilised methacholine chloride (*Provocholine*, Hoffman La Roche, Basel, Switzerland) in local centres, either by pharmacy departments using standard procedures or by technicians using the agreed study protocol.

**Urinary electrolytes**

Urine was collected over a 24 hour period from male subjects only, and aliquots were taken for the measurement of sodium, potassium, calcium, magnesium and creatinine. Specimens were collected over the weekend, if this was convenient to the subjects, as this has been shown to give equivalent results to collections taken during the week. Analyses were performed in a central laboratory (Professor Kesteloot, Leuven).

**THE EXECUTION OF THE STUDY**

Prior to data collection investigators from each of the centres attended a series of training seminars in which the protocols were explained and the standardised techniques demonstrated. Subsequently there has been an extensive quality control procedure in the study. This has involved:

a) Visits by members of the central coordinating team to two centres in each region with subsequent visits by members of those regional centres to the other centres in the region. These visits have checked that the protocol is being followed and have noted any deviations from the protocol.
b) Assessment of within-observer variation in results from skin testing with histamine Phazets. Fieldworkers had to achieve a set standard before being allowed to undertake these tests on study subjects.

c) Monthly checks on the output of the nebulisers used for challenge tests.

d) HPLC assays of the methacholine solutions from the different centres, undertaken in a central laboratory to check the concentrations.

THE ANALYSIS

The following initial analyses will be undertaken:

1. The distribution of symptoms and bronchial lability will be studied in relation to age, sex, smoking history, mean skin wheal diameter to all allergens, sodium excretion (after allowing for the confounding effects of height and creatinine excretion), with and without allowing for the independent effects of country and area.

2. The distribution of skin sensitivity and serum IgE will be assessed in relation to age, sex, smoking, the mother's and father's smoking histories in the first instance, with and without allowing for the independent effects of country and area.

3. Supplementary analyses will be run to test which other risk factors are associated with symptoms / bronchial hyperreactivity or with skin sensitivity / serum IgE. These factors will include housing conditions, occupation, ownership of pets, diet, family structure as a proxy for early exposure to infections, and the use of medications.

If independent country or area effects are still present at this stage information on pollution levels, population density and climate, if available, will be tested for an association with the dependent variables.
Organisational structure

COORDINATING CENTRE

Project Leader: Dr P Burney
Statistician: Miss S Chinn
Epidemiologist: Dr D Jarvis
Coordinator: Dr C Luczynska

Address: Department of Public Health Medicine, UMDS St Thomas' Campus, Lambeth Palace Road, London SE1 7EH, United Kingdom

PROJECT MANAGEMENT GROUP

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LIST OF PARTICIPATING CENTRES

EC Member States

P Vermeire (Antwerp/South Antwerp); R Dahl (Aarhus); H Magnussen (Hamburg); H Wichmann (Erfurt); N Papageorgiou (Athens); J Anto (Barcelona); A Capelastegui (Galdakao); J Castillo (Sevilla); J Maldonado (Huelva); J Moratalla (Albacete); R Quiros (Oviedo); J Bousquet (Montpellier); F Neukirch (Paris); I Pin (Grenoble); A Taytard (Pessac); D Teculescu (Nancy); J Prichard (Dublin/Kilkenny); M Bugiani (Torino); R De Marco (Pavia); V Lo Cascio (Verona); B Rijcken (Bergen-op-Zoom/Geleen/Groningen); R Avila (Lisboa); C Loureiro (Coimbra); A Marques (Porto); M Burr (Cardiff); R Hall (Ipswich); B Harrison (Norwich); J Stark (Cambridge); C Florey (Dundee).

COST Countries

W Popp (Wien); T Gislason (Gardabaer); A Gulsvik (Bergen); U Ackermann-Liebrich (Basel); N Lindholm (Goteborg); G Boman (Uppsala); L Rosenhall (Umea).

Centres taking part at own expense

N Ait-Khaled (Algiers); M Abramson (Melbourne); J Manfreda (Winnipeg and 5 other centres in Canada); R Chowgule (Bombay); J Crane (Wellington and 3 other centres in New Zealand); A Al-Frayh (Riyadh); I Stepanov (Riga); S Buist (Portland)
References


29. Magnusson CG. Maternal smoking influences cord serum IgE and IgD levels and increases risk of subsequent infant allergy. J Allergy Clin Immunol 1986; 78:898-904


37. Burney PGJ, Chinn S. Developing a new questionnaire for measuring the prevalence and distribution of asthma. Chest 1987; 91:795-835


44. Quanjer PhH. Standardised lung function testing. Bull eur Physiopath Respir 1983; suppl 5
ALLERGY TESTING

Introduction

Each subject is to be tested for allergy by measurement of serum specific IgE and skin sensitivity to common allergens.

Blood collection for serum analyses

A blood sample is collected from each volunteer and an aliquot of serum is sent to a central laboratory at Kabi Pharmacia Diagnostics, Uppsala. This is analysed for total IgE and specific IgE to a panel of common environmental allergens, which include Dermatophagoides pteronyssinus, cat, grass, Cladosporium and one of the following local allergens: birch (northern Europe), Parietaria (southern Europe) and ragweed (USA).

Skin prick testing

Skin prick testing is carried out using Phazets (Kabi Pharmacia Diagnostics AB), which are stainless steel lancets pre-coated with lyophilised standardised allergen extracts. The Phazets should be stored at 4°C and have a shelf life of three years.

Each subject is skin tested using the following panel of allergens:

d1 D PTERONYSSINUS
e1 CAT
g6 TIMOTHY GRASS
m2 CLADOSPORIUM HERBARUM
m6 ALTERNARIA ALTERNATA
t3 BIRCH
t9 OLIVE (O EUROPEA)
w1 COMMON RAGWEED
w21 PARIETARIA JUDAICA

positive control: HISTAMINE
negative control: UN-COATED PHAZET

In addition, each centre can add up to two locally important allergens.
Serum total and specific IgE

The following materials are recommended for blood collection and the transportation of serum samples:

- Glass blood collection tubes with no anticoagulant (e.g. Vacutainer tube Becton Dickinson Cat No 6430)
- 2 mL polypropylene Micro-tubes with screw caps (Sarstedt Cat No 72 694)
- Indelible (waterproof) pens for labelling (Sarstedt Cat No 95 954/3)
- Styrofoam boxes (hold 100 tubes) (Sarstedt Cat No 95 064 249)

10 mL of venous blood is taken from each subject and left to coagulate either for 3-6 hours at room temperature or overnight at 4°C, but preferably no longer than this to avoid excessive haemolysis. The blood is centrifuged at 2500-3000 rpm for 10-15 minutes, the serum pipetted off and aliquoted into 2 mL Sarstedt tubes. At least two aliquots are required, one for analysis by Kabi Pharmacia and spare aliquots in case of a loss of samples. Any further aliquots may be used for local projects. Aliquots for testing are stored at -20°C, packed in Styrofoam boxes and sent in dry ice to the Central Laboratory at Kabi Pharmacia at regular intervals throughout the study period.

Skin testing using Phazets

In addition to the Phazets, a skin test grid for application of Phazets (provided by Kabi Pharmacia), transparent tape at least 25 mm wide, ball-point pen or fine felt tip pen, and a timer are also required.

Phazets are always applied in exactly the same order and the results are transferred in the same orientation to the data collection sheet. The fieldworker should carry out the skin testing according to the following instructions:

1. Place skin 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. Open the Phazet envelope by tearing off at the perforated line and remove the lancet without touching the allergen-coated tip.

3. Hold the lancet at 90° to the skin and with the forefinger press against the skin for at least 1 second. Always apply the same pressure.

4. Remove the lancet with an upward motion and discard.

5. Remove the skin test grid.

6. Read the result after 15 minutes by drawing round the perimeter of the wheal with a ballpoint pen or fine felt-tip pen. Always draw in the same order as the application of the Phazets.

7. Press a strip of transparent tape against the skin and transfer the prints to the grid on the data collection sheet.

8. Measure the wheal diameter (mm) at its widest point and at 90° to the diameter at the mid-point and record both diameters to the nearest whole millimetre on the data collection sheet.

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

**Skin testing training for fieldworkers**

Each fieldworker must undergo the specified training in order to perform skin tests consistently and in a standardised manner. Before starting the study, the fieldworker should perform two histamine skin tests on each of 20 volunteers (total 40 tests done by each fieldworker). Trained fieldworkers should have a coefficient of variation (CV) of less than 30%.
The coefficient of variation of each fieldworker is carried out as follows:

Calculate the log to base e of each mean wheal diameter recorded in mm.

If there are exactly two skin tests carried out on each volunteer:

Use the following formula to calculate the CV:

\[
CV = \sqrt{\frac{\sum(d^2/2)}{n}} \times 100 \%
\]

where
- \(d\) = difference between two \(\log_e\) values for each volunteer
- \(n\) = number of volunteers

If there are not exactly two skin tests for each volunteer:

A between volunteer 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 (%).
**ALLERGY SKIN TEST TRAINING SHEET**

Centre: _____________________

Fieldworker Name: _____________________

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

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### CALCULATION OF CV IF 2 TESTS CARRIED OUT ON EACH VOLUNTEER:

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\[ \text{Coefficient of variation} = \sqrt{\frac{\sum d^2}{d}} \times 100\% \]

where \( d \) = diameter
\[ \log_e = \log \text{ to base } e \]
\[ d = \log_e(\text{mean diam 1}) - \log_e(\text{mean diam 2}) \]
\[ n = \text{number of volunteers} \]

**Reference:**
TWENTY FOUR HOUR URINE COLLECTION

Introduction

Urine samples are collected from male subjects only. Samples of urine are sent to the Central Laboratory in Leuven for the determination of 24 hour excretion of sodium, potassium, calcium, magnesium and creatinine.

Materials required

- 24 hour plastic urine containers (2.5 L)
- 2 L measuring cylinder
- Boric acid (Analar Reagent quality; min 99.5% pure)
- 10 mL plastic tubes with screw caps

Preparation of collection bottles

Each testing centre will require at least one hundred 2.5 litre plastic 24 hour urine containers which will be recycled during the period of the study. The plastic containers should be washed with a mild detergent and then thoroughly rinsed with warm water. Those that retain staining or odour of urine should be discarded. After cleaning, one tablespoon (10-15 g) of BORIC ACID should be added to each container. A blank label is affixed for the subjects personal number and date when the container is issued.

Clinic visit

Male volunteers will be requested to complete a 24 hour urine collection. The urine collection may be started during the clinic visit or the following morning, AFTER emptying the bladder. Alternatively, the collection can be carried out over the weekend. Clear written instructions must be given to the volunteers. They will be given two 2.5 litre plastic containers and an opaque plastic bag. The subject’s personal number and the date should be written in indelible ink on the labels of each container. All subjects will be reminded to empty their bladder prior to a bowel movement and to ensure that the bottle is always held during collection to avoid
spilling. When the urine collection is complete the filled bottles must be returned to
the clinic as soon as possible within 24 hours after completion.

Aliquoting urine samples

The total volume of urine collected is measured using a graduated measuring

cylinder and recorded prior to aliquoting. Four 8 mL aliquots should be taken from
each 24 hour collection and placed in 10 mL plastic tubes with screw caps, leaving at
least 2 cm unfilled space. Each aliquot is labelled with the area number, the subject’s
personal number and the date.

Storage of samples

Twenty four hour urine collections must be stored in a refrigerator at or below 4°C
as soon as possible, and preferably within 24 hours of completion. Aliquots must be
taken as soon as possible and then frozen at -20°C. Samples should remain frozen
until analysed in Belgium. Two samples will be sent to Belgium and two will be kept
frozen at the local centre as back-up specimens in case of loss or leakage in transit.

Random repeat samples

Up to 25 urine samples should be repeated at random from the subjects who have
provided a urine sample at the end of the study period.
The European Community Respiratory Health Survey

LUNG FUNCTION TESTS

CRITERIA FOR TESTING

Criteria for baseline spirometry

The purpose of baseline spirometry is to record an accurate Forced Expiratory Volume in one second (FEV₁) and Forced Vital Capacity (FVC) from every subject who attends the testing centre.

ACCEPTANCE CRITERIA:

Any subject who is able to attend the testing centre.

EXCLUSION CRITERIA:

If the subject smokes: Lung function testing should be carried out at least one hour after the last cigarette has been smoked.

If the subject has used an inhaler: Lung function testing should be carried out at least one hour after the use of any inhaler.

If the subject has used an inhaler that is not a beta-2-agonist or an anticholinergic inhaler in the last one to four hours: Lung function testing is carried out and the data recorded.

If the subject has used an inhaler that is a beta-2-agonist or an anticholinergic inhaler in the last one to four hours: If the subject is willing to come back another time for lung function testing, another appointment should be made. If the subject is unable or reluctant to return another time, testing should proceed and the medication used should be recorded.

If the subject has taken an oral beta-2-agonist or an oral theophylline or an oral antimuscarinic within the last eight hours: If the subject is willing to come back another time for lung function testing, another appointment should be made. If the subject is unable or reluctant to return another time, testing should proceed and the medication used recorded.

If the subject has had a respiratory tract infection in the last three weeks: Another appointment should be made unless the subject is unwilling to come back, in which
case testing should continue. The number of days elapsed since the end of the respiratory infection should be recorded.

If, after a total of nine attempts, a subject is unable to produce a technically satisfactory manoeuvre, no FEV$_1$ or FVC will be recorded.

**Expected FEV$_1$ values**

Normal FEV$_1$ values will be calculated using the following equations:

- **Males:** $4.30 \times H - 0.029 \times A - 2.49$
- **Females:** $3.95 \times H - 0.025 \times A - 2.60$

where

- $H =$ height in metres
- $A =$ age in years (range 25-44).

These equations are only valid for subjects over the age of 25. Subjects aged 20-24 should have their expected FEV$_1$ calculated as if their age is 25.

**Criteria for methacholine challenge**

The aim of methacholine challenge is for subjects to inhale increasing concentrations of methacholine solutions and to monitor any change in FEV$_1$ by repeated spirometric testing. The cumulative dose of methacholine required to produce a 20% fall in FEV$_1$ from the control (post-diluent) level will be calculated (PD$_{20}$).

**ACCEPTANCE CRITERIA:** Any subject who fulfils all three of the following criteria is accepted:

1) is able to perform satisfactory FEV$_1$ and FVC manoeuvres,
2) has signed a consent form for methacholine challenge,
3) is not in the categories for exclusion (see below).

**EXCLUSION CRITERIA:** Any subject who fulfils any one of the following criteria is excluded:
The European Community Respiratory Health Survey

1) has had a heart attack in the last three months,
2) has any heart disease for which he/she is taking medication,
3) has epilepsy for which he/she is taking medication,
4) is pregnant,
5) is breast feeding,
6) is taking a beta-blocker for any reason (including eye drops).

These criteria will be assessed by the Lung Function Questionnaire.

In addition, any subject who fulfils either of the following is excluded:

7) has an FEV$_1$ less than 70% of the mean predicted value,
8) has an FEV$_1$ less than 1.5 litres.
These are assessed during the baseline spirometry.

**Criteria for bronchodilator challenge**

The FEV$_1$ and FVC will be measured following the administration of salbutamol by metered dose inhaler (MDI) via a Volumatic spacer.

**ACCEPTANCE CRITERIA:** Any subject who fulfils all of the following criteria is accepted:

1) has produced technically satisfactory FEV$_1$ and FVC manoeuvres,
2) refuses to undergo or is excluded from methacholine challenge,
3) has signed a consent form for bronchodilator challenge,
4) is not excluded by the following exclusion criteria.

**EXCLUSION CRITERIA:** Any subject who fulfils any one of the following criteria is excluded:

1) has had a heart attack in the last three months,
2) has any heart disease for which he/she is taking medication,
3) has epilepsy for which he/she is taking medication,
4) is pregnant,
5) is breast feeding,
6) is taking a beta-blocker for any reason (including eye drops).
These conditions will be assessed by the Lung Function Questionnaire.

**Making the appointment for testing**

*Ideally, lung function testing should be performed:*

1) more than four hours after the use of a beta-2-agonist or anticholinergic inhaler,
2) more than eight hours after oral beta-2-agonist or theophylline or oral antimuscarinic.

*When the appointment for lung function testing is made the fieldworker should determine if the subject is taking any of the following medications:*

1) beta-2-agonist inhaler,
2) anticholinergic inhaler,
3) oral beta-2-agonist,
4) oral theophylline,
5) oral antimuscarinic.

If the subject is taking any of these medications (or any other inhaler) an appointment time should be agreed that will cause the least disruption to the subject's normal dosing schedule.

*One simple way of ensuring compliance with these instructions is to:*

1) avoid early morning appointments for those using inhalers,
2) fix a time for an appointment and then ask the subject to take their inhalers four hours before and oral medication eight hours before testing.

The fieldworker should ensure that the subject has not had a respiratory tract infection in the three weeks prior to testing and should advise the subject not to smoke for one hour prior to coming to the testing centre. A letter should be sent to the subject explaining this.

**Subjects who have not followed guide-lines**
Those who have had a cigarette in the last hour should have the lung function test delayed until one hour has elapsed. (Most subjects will be in the centre for at least one hour.)

Those who have had an inhaler in the last four hours or oral medication in the last eight hours may fall into one or more of the following categories:

1) misunderstood the instructions,
2) forgot the instructions,
3) ignored the instructions,
4) may have symptoms too severe to follow the instructions.

Lung function testing may still be carried out unless the subject is excluded for other reasons, and recent medication should be noted in the Lung Function Questionnaire.
THE FORCED EXPIRATORY MANOEUVRE

General guide-lines

All forced expiratory manoeuvres will be performed:

1) sitting,
2) with noseclip on,
3) using a plastic or cardboard mouthpiece without teethgrips,
4) tight clothing should be loosened.

Two types of forced expiratory manoeuvre will be used in this protocol:

1) During baseline spirometry and bronchodilator challenge FVC will be measured and all subjects must exhale fully.

2) During methacholine challenge only the FEV₁ needs to be recorded and the technician may interrupt the exhalation when this has been achieved.

A technically unsatisfactory manoeuvre (FEV₁ or FVC) is defined as:

1) an unsatisfactory start of expiration characterised by excessive hesitation or false start,
2) coughing during the first second of the manoeuvre, thereby affecting the measured FEV₁ value, or any cough that interferes with the accurate measurement of FVC,
3) Valsalva Manoeuvre (glottis closure),
4) a leak in the system or around the mouthpiece,
5) an obstructed mouthpiece, e.g. the tongue in front of the mouthpiece.

Manoeuvres which have these faults are technically unsatisfactory and are rejected as failed attempts.
Evidence of poor compliance is shown by:

1) greater than 5% variation in FEV₁ between blows,
2) greater than 150 mL or 5% FVC back-extrapolated volume,
3) peak expiratory flow that is less than 85% of the best recorded,
4) expiratory time that is less than six seconds.

If these features are noted technicians should encourage the subject to produce a better reading but the blows should not be excluded as failed attempts on these criteria alone.

A manoeuvre may only be rejected as a failed attempt if it is 'technically unsatisfactory'. Manoeuvres with evidence of 'poor compliance' only should not be rejected.

Instructions to subjects

Some of the subjects will never have used any form of lung function testing equipment before and others will be very familiar with it.

Technicians should explain to the subject that the aim of the test is to find out how much air can be blown out of the lungs and how forcefully it can be blown out.

This can be done by asking the subject to follow these steps:

1) Take in as deep a breath as possible and when full -
2) Place the mouthpiece in his/her mouth.
3) Close his/her lips tightly around the mouthpiece.
4) Blast or blow out through the mouthpiece into the spirometer blowing air out as hard, fast, smoothly and completely as possible.

The subject should continue to push out air actively for as long as possible (FVC manoeuvre) or until the technician tells him/her to stop (FEV₁ manoeuvre). During this time the technician must offer positive encouragement to push or squeeze out more air.
Baseline spirometry

1) Ensure that it is appropriate to perform lung function testing.

2) Demonstrate the manoeuvre to all subjects at least once (more often if he/she appears uncertain).

3) Ask the subject to carry out five FVC manoeuvres.

4) Record the FEV₁ and FVC and Peak Expiratory Flow (in litres per second) from at least two and up to five technically satisfactory manoeuvres.

5) If the subject has failed to produce two technically satisfactory manoeuvres after five attempts, the technician should show them again how to conduct the manoeuvre and allow them four more attempts.

6) Any subject who is unable to produce two technically satisfactory manoeuvres after nine attempts should not be tested further and no FEV₁ / FVC data should be recorded.

7) The number of rejected attempts should be recorded as appropriate on the Lung Function Data Collection Sheet.

Methacholine challenge

During methacholine challenge the subject may need to perform 30 or more expiratory manoeuvres and, to minimise exhaustion, the forced expiration will be abandoned each time after one second when the FEV₁ has been recorded.

1) Two minutes after inhalation from the dosimeter up to five attempts should be made to record an FEV₁.

2) As soon as two technically satisfactory manoeuvres have been achieved these readings are recorded. The next dose can be given as soon as possible after the completion of these measurements.
3) Further testing should be abandoned if the subject is unable to produce two technically satisfactory manoeuvres within five attempts.

If a reversal of bronchoconstriction needs to be carried out then the procedure is the same as the bronchodilator challenge.

**Bronchodilator challenge**

A bronchodilator challenge will be given to those who do not undergo methacholine challenge. Any subject who has more than a 10% fall in FEV$_1$ from baseline during the methacholine challenge test should have their bronchoconstriction reversed at the end of the test and before leaving the test centre, by the same method.

This should be carried out by two inhalations of 100 µg each of salbutamol from a metered dose inhaler (MDI) via a Volumatic spacer. Subjects who are known asthmatics and familiar with inhaler and Volumatic usage can self-administer this dose.

The fieldworker should shake the MDI, place it into the end of the Volumatic and fire once.

_The subject should then:_

1) Exhale to functional residual capacity.
2) Place lips around Volumatic and inhale deeply and slowly.
3) Hold breath for at least 15 seconds.
4) Exhale.

The inhaler should be fired again and steps 1 - 4 repeated.

The FEV$_1$ and FVC are measured 10 minutes after the administration of bronchodilator. During the bronchodilator challenge FVC manoeuvres will be used. Up to nine attempts may be made to obtain two technically satisfactory recordings after the inhalation of bronchodilator.
THE METHACHOLINE SOLUTIONS

Source and supply

Methacholine (Provocholine) will be obtained from Hoffman La Roche in 5 mL vials containing 100 mg lyophilised methacholine chloride.

The diluent

Saline, buffered with phosphate to obtain physiological pH, can be used as the diluent. Phenol must not be used as a preservative because of concerns regarding its safety. No other preservative may be used. Citric acid / citrate buffer must not be used.

The recommended formula for the diluent is as follows:

Methacholine chloride diluent physiological pH range 7.2-7.4:

1000 mL normal saline (0.9% NaCl)
3 mL neutral sodium phosphate solution

where neutral sodium phosphate solution is

23.6 g Na₂HPO₄·12H₂O and
3.04 g NaH₂PO₄·2H₂O

made up to 100 mL with distilled water.

Preparation of methacholine solutions

Where possible, centres are advised to have the methacholine solutions prepared by a pharmacy using aseptic or sterile conditions. Otherwise, the following procedure should be used for making up the dilutions.
The European Community Respiratory Health Survey

*Methacholine solutions to be made up:*

BLACK 12.5 mg/mL
RED 6.25 mg/mL
YELLOW 1.56 mg/mL
BLUE 0.39 mg/mL
GREEN 0.195 mg/mL
WHITE diluent

1) 4 mL of diluent is added to the vial containing 100 mg methacholine using a 'Class A' graduated glass pipette (makes 25 mg/mL).

2) Transfer 2 mL of the 25 mg/mL solution to 2 mL of diluent in a vial to make a 12.5 mg/mL solution (BLACK).

3) Transfer 2 mL of the 25 mg/mL solution to 6 mL of diluent in a vial to make a 6.25 mg/mL solution (RED).

4) Transfer 2 mL of the 6.25 mg/mL solution to 6 mL of diluent in a vial to make a 1.56 mg/mL solution (YELLOW).

5) Transfer 2 mL of the 1.56 mg/mL solution to 6 mL of diluent in a vial to make a 0.39 mg/mL solution (BLUE).

6) Transfer 2 mL of the 0.39 mg/mL solution to 2 mL of diluent in a vial to make a 0.195 mg/mL solution (GREEN).

This dilution procedure provides 4 mL of the 12.5 mg/mL, 6 mL of each of the 6.25, 1.56 and 0.39 mg/mL, and 4 mL of the 0.195 mg/mL solutions. It can be scaled up as necessary to provide larger volumes.

For maximum accuracy a 'Class A' glass pipette should be used for the initial dilution of methacholine, but 'Eppendorf' pipettes can be used subsequently. All pipettes should be calibrated once a month using standard techniques.
Storage and shelf life of methacholine solutions

All solutions must be kept refrigerated at 4°C in sealed containers. The shelf life of methacholine solutions is six weeks if they are stored under aseptic conditions. Solutions made up non-aseptically should be discarded within 48 hours. Solutions from the nebulisers must not be replaced in the container but should be discarded after use.

Session number and order in session

Each time the nebulisers are filled with fresh methacholine solution a new session of testing is said to have started. Each session should be sequentially numbered. Each challenge within each testing session should also be sequentially numbered and recorded on the Lung Function Data Collection Sheet. The numbering system depends on which methacholine protocol is used.

Method 1

At the beginning of a session all nebulisers contain 3 mL methacholine. Twelve subjects are tested and their order in session is 1-12. After the 12th person has been tested all solutions are discarded and the nebulisers are cleaned. The next session begins when new solutions are added. A session may be extended over one night only by placing the nebulisers containing solutions upright in the fridge, covered with parafilm.

Method 2

At the beginning of a session all nebulisers contain 3 mL methacholine. Six subjects are tested and their order in session is 1-6. After the 6th person has been tested the 12.5 mg/mL solution is discarded, the nebuliser is cleaned and dried, and 3 mL of fresh 12.5 mg/mL solution is added. Six more subjects are tested and they are numbered 7-12. After the 12th person has been tested all solutions are discarded and the nebulisers are cleaned. The next session begins when new solutions are added. A session may be extended over one night only by placing the nebulisers containing solutions upright in the fridge, covered with parafilm.
THE MEFAR MB3 DOSIMETER

Quality control of Mefar dosimeter nebuliser output

The methacholine challenge protocol has been written assuming that each single inhalation delivers approximately 0.01 mL solution to the mouth. The Mefar nebulisers are calibrated by the manufacturer, but to ensure the accuracy of calibration and to overcome the possibility of a reduction in performance during the period of the test, the actual output of the nebulisers must be measured every month and recorded.

Each nebuliser should be colour coded for the solution it will contain as follows:

1) BLACK  12.5 mg/mL
2) RED  6.25 mg/mL
3) YELLOW  1.56 mg/mL
4) BLUE  0.39 mg/mL
5) GREEN  0.195 mg/mL
6) WHITE  diluent

To measure nebuliser output:

1) Set the Mefar so that it will automatically fire (adjust the thermistor sensitivity).

2) Set the other variables - number of inhalations 10, pause time 6 seconds, inhalation time 1 second.

3) Place 3 mL of distilled water into each nebuliser.

4) Weigh the nebuliser and record the weight in mg (weight 1).

5) Connect each nebuliser to the dosimeter and simulate 10 inhalations.

6) Re-weigh each nebuliser and record the weight in mg (weight 2).

7) Repeat step 5 and 6 (weight 3).
8) Calculate volume of each inhalation using:

\[
\text{weight 1 - weight 2} = \frac{\text{mL/inhalation}}{10}
\]

\[
\text{weight 2 - weight 3} = \frac{\text{mL/inhalation}}{10}
\]

Divide the two values by two to obtain the average output.

9) Repeat procedure for each nebuliser.

The room temperature at the time of calibration should be recorded on the calibration sheet.

**Setting up the Mefar dosimeter**

3 mL of methacholine solution should be placed in the appropriate nebuliser. A dry sterile mouthpiece should be connected for each new subject.

*The Mefar should be set at:*

1) inhalation time: 1 second
2) pause time: 6 seconds

**The standard inhalation**

*The sequence of inhalation is:*

1) Slow expiration to functional residual capacity.
2) Place lips around mouthpiece to produce airtight seal.
3) Slow inspiration to total lung capacity.
4) Hold breath for at least three seconds.
5) Remove mouthpiece and exhale.
The procedure is repeated after six seconds until sufficient inhalations for the dose have been performed. Inhalations may be performed on consecutive breaths if desired. Spirometric testing is carried out two minutes after the dose. As soon as two FEV$_1$ manoeuvres have been recorded, the test is continued with the next dose.

The end of the testing session

Solutions remaining in the nebulisers must be discarded and under no circumstances should they be returned to the storage containers. All nebulisers must be cleaned and dried. All mouthpieces must be cleaned, sterilised and thoroughly rinsed to ensure that there is no sterilising solution left on the surface.
THE METHACHOLINE PROTOCOL

Instructions for baseline spirometry

Perform full FVC manoeuvres as described previously for 'Baseline spirometry' (The forced expiratory manoeuvre). Record INITIAL FEV\textsubscript{1} and FVC. Calculate the BEST INITIAL FEV\textsubscript{1} as a percentage of the total predicted.

Measurement of control (post-diluent) FEV\textsubscript{1}

The control FEV\textsubscript{1} is the FEV\textsubscript{1} measured following the inhalation of diluent. Four inhalations of diluent (WHITE nebuliser) are given, as described in 'The standard inhalation'.

Perform FEV\textsubscript{1} manoeuvres as described in 'Methacholine challenge' (The forced expiratory manoeuvre). Record CONTROL (POST-DILUENT) FEV\textsubscript{1}. Calculate BEST CONTROL FEV\textsubscript{1} as a percentage of the BEST INITIAL FEV\textsubscript{1}.

If the BEST CONTROL FEV\textsubscript{1} is less than 90\% of the BEST INITIAL FEV\textsubscript{1} methacholine challenge is not carried out. Bronchoconstriction should be reversed by administering 200 µg salbutamol by MDI via a Volumatic and full FVC manoeuvres should be repeated.

If the BEST CONTROL FEV\textsubscript{1} is within 10\% of the best initial FEV\textsubscript{1}. Calculate 80\% of the BEST CONTROL FEV\textsubscript{1}. Calculate 90\% of the BEST CONTROL FEV\textsubscript{1}. Methacholine challenge may now be conducted following either the short or long protocol.

Choice of methacholine protocol (Method 1 or Method 2)

One of two methacholine challenge protocols may be used, with a choice in each of a long or short protocol. Each centre should decide whether to use Method 1 or 2 as they differ in the dose at which the challenge is started and the maximum dose given. As two methods may be used it is essential that the correct box numbers are attributed to each set of measurements. It is also essential that the correct system for the order in session and the session number is used.
Choice of long or short protocol

Each subject can be challenged on the short or long protocol. The long protocol will increase by doubling doses and the short by quadrupling doses. Subjects most likely to react to methacholine should be tested on the long protocol. Subjects who are unexpectedly reactive and have been allocated to the short protocol may switch to the long protocol during the challenge to avoid severe bronchoconstriction. The choice of protocol for each subject will be assessed by the Main Questionnaire. The questions to be used to direct subjects to the long protocol may be decided locally, but the following are recommended:

Subjects who answered 'YES' to any one of Questions 1, 2, 3, 5, 11 or 13 in the Main Questionnaire, that is any subject who has:
1) had wheezing or whistling in their chest in the last 12 months (Q1),
2) woken with tightness of chest in the last 12 months (Q2),
3) had an attack of shortness of breath during the day while at rest in the last 12 months (Q3),
4) been woken by an attack of shortness of breath in the last 12 months (Q5),
5) trouble with their breathing (Q11),
6) ever had asthma (Q13).

Methacholine challenge protocol (Table)

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Each centre must choose between Methods 1 and 2. Each centre must decide which questions on the Main Questionnaire will direct subjects to the short or long protocols.

**Changing from short to long protocol**

If, during the short protocol, the FEV$_1$ falls 10% or more from the best control FEV$_1$, the subject should change protocol and receive the next dose level on the long protocol.

For example: Method 1. A subject following the short protocol shows a fall of 10% after Dose 4 (three inhalations of 0.39 mg/mL). They should inhale Dose 5 (one inhalation of 1.56 mg/mL) next.

*Short protocol:*

Change to long protocol if FEV$_1$ falls below 90% of the BEST CONTROL FEV$_1$. Go to next dose level on long protocol.

STOP challenge if FEV$_1$ falls below 80% of the BEST CONTROL FEV$_1$

*Long protocol:*

STOP challenge if FEV$_1$ falls below 80% of the BEST CONTROL FEV$_1$

**Completion of test**

The methacholine challenge is complete when a cumulative dose of 1 mg (Method 1) or 2 mg (Method 2) of methacholine has been reached.

*It is stopped sooner if:*

1) there is greater than 10% fall in FEV$_1$ from the **BEST BASELINE FEV$_1$** following inhalation of diluent,
2) there is greater than 20% fall in FEV₁ from the BEST CONTROL FEV₁ following inhalation of any concentration of methacholine solution,

3) the subject is not able to perform two technically satisfactory manoeuvres in five attempts following any dose level,

4) the subject does not wish to carry on.

Subjects may complain of mild chest tightness, coughing or wheezing but if lung function does not demonstrate a 20% fall in FEV₁ this is not an indication to stop the test.

**Reversal of bronchoconstriction**

Two inhalations of 100 µg salbutamol are administered by MDI as described in 'Bronchodilator challenge' (The forced expiratory manoeuvre). Perform full FVC manoeuvres as described in 'Methacholine challenge'.

Record the POST-BRONCHODILATOR FEV₁ and FVC.

Calculate the BEST POST-BRONCHODILATOR FEV₁ as a PERCENTAGE of the BEST INITIAL FEV₁.

If the best post-bronchodilator FEV₁ is more than 90% of the best initial FEV₁ the test is over.

*EACH CENTRE SHOULD PREPARE PROTOCOLS TO BE FOLLOWED IN THE EVENT OF A SUBJECT NOT RETURNING TO WITHIN 10% OF THE BASELINE.*

**BRONCHODILATOR CHALLENGE PROTOCOL**

Two inhalations of 100 µg salbutamol are administered by MDI as described in 'Bronchodilator challenge'. Perform full FVC manoeuvres as described in 'Baseline spirometry'. Record the POST-BRONCHODILATOR FEV₁ AND FVC.
Each pipette used should be quality controlled at a relevant volume once a month by weighing out 20 aliquots of water. The pipette used for dispensing diluent into the methacholine vial should be tested at 4 mL. Weights should be recorded in mg to three decimal places.

For each pipette work out the mean volume, mean error, relative error and CV as follows:

**MEAN VOLUME = \( \frac{\sum \text{all volumes}}{20} \)**

**MEAN ERROR = mean volume - expected volume**

**RELATIVE ERROR = mean error as % of expected volume**

**CV = \frac{\text{standard deviation}}{\text{mean}} \times 100\%**  
(Typical values 5 mL pipette: Relative error <±1.5%, CV < 0.3%)

<table>
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<tr>
<th>Pipette no:</th>
<th>ALIQUOTS</th>
<th>VOLUME (mL)</th>
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The European Community Respiratory Health Survey

### RECORD OF NEBULISER OUTPUTS

Centre: ____________________

Area number

Fieldworker name:

Date

Fieldworker number

The output of the nebulisers must be calculated each month using the following method:

Place 3 mL of distilled water in the nebuliser.
Weigh on scales accurate to 0.0001 g and round to the nearest 0.001 mg (weight 1).
Simulate 10 inhalations and re-weigh (weight 2).
Simulate 10 inhalations and re-weigh (weight 3).
Calculate the volume of each inhalation and then the average.

Nebuliser output must be measured every month and recorded on this sheet.
The room temperature at the time of calibration should be measured and recorded below.

This sheet should then be returned to the Coordinating Centre.

The exact output of the nebuliser will be incorporated into the calculation of the PD$_{20}$.  

<table>
<thead>
<tr>
<th>NEBULISER</th>
<th>WHITE Diluent</th>
<th>GREEN 0.195 mg</th>
<th>BLUE 0.39 mg</th>
<th>YELLOW 1.56 mg</th>
<th>RED 6.25 mg</th>
<th>BLACK 12.5 mg</th>
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<tbody>
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<td>weight 1</td>
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### The European Community Respiratory Health Survey

<table>
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<th>weight 3-2</th>
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<td>Average mL per inhalation</td>
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Room temperature (°C): [ ]