ISAAC

INTERNATIONAL STUDY OF ASTHMA AND ALLERGIES IN CHILDHOOD

# Phase II Modules

Münster, Germany

May 1998

# CONTENTS

# STUDY PROTOCOL

Following the worldwide success of ISAAC Phase I, it is anticipated that a number of further studies will be undertaken throughout the world to address hypotheses of interest which arise from the Phase I results. This document outlines one specific proposal developed by the ISAAC Steering Committee to promote internationally standardised comparisons of disease and relevant risk factors using the archive of methods developed over the past few years by ISAAC collaborators. Investigators who are planning similar geographical comparisons within their region are encouraged to consider a similar methodology, but are free to choose a different approach to study design, sample selection or fieldwork. In contrast to ISAAC Phase I, a worldwide co-ordination and data centre for Phase II studies is not funded at present.

## Sampling strategies

### Selection of schools

A simple random sample of at least 10 schools will be chosen from a complete sampling frame of all schools in a defined geographical area (the ISAAC centre). The number of schools will be determined by the required sample size (see below). Size of school will not be a consideration in sampling. Schools which do not agree to take part will be replaced by another school selected at random. The sampling strategy and school participation rate must be recorded.

### Choice of age group

The school year studied will be that in which the majority of children are aged 10 years 0 months to 10 years 11 months at the start of the fieldwork. This is a different age group from those involved in previous ISAAC surveys in the same centres, thus offering an independent confirmation of prevalence differences. This age group has been chosen because 10-year-olds are known to participate and perform satisfactorily in all proposed tests, including spirometry and blood sampling (often problematic in younger age groups) and examination for flexural dermatitis (problematic in older children in some cultures).

## Selection of children

Two alternative options are recommended. One option should be chosen, depending upon the availability of local resources:

Option A: All children in the selected schools will take part in all tests, provided that parental consent is obtained.

Option B: All children will be examined for flexural dermatitis and have skin prick tests, but other tests will be restricted to subgroups of wheezy and non-wheezy children.

**Option A:** A survey will be conducted among at least 2000 children in each centre using the ISAAC written questionnaires for parental completion. In areas of low wheezing prevalence, as determined from the Phase I results, a larger sample size of up to 3000 children will be required to generate at least 100 children with wheezing in the past year. All children in the eligible class in the selected schools will be included in this initial survey. On the conservative estimate of 50% response with parental consent to clinical tests, at least 1000 children will be eligible for inclusion in further tests. The response rate and parental consent rate should be recorded.

**Option B:** The initial survey will proceed as in option A, but subgroups of 200 children in each centre will be selected, stratified by the presence of wheeze in the past year, as reported by parents in the initial questionnaire. The subgroups will comprise:

i) At least 100 children chosen at random from those with a history of wheeze in the past year, irrespective of whether asthma has been diagnosed.

ii) At least 100 children selected at random from all those without wheeze in the past year. These are likely to include some children with a history of wheeze ever, but not in the past year, and may include some children with a diagnosis of asthma, but no wheeze in the past year.

This stratified sampling procedure will increase the proportion of those tested who have a history of wheezing,

thus permitting comparisons between centres of the patterns of bronchial responsiveness among children reported to wheeze, as well as providing a weighted prevalence estimate of the prevalence of BHR in the whole population. It also has the practical advantage of reducing the time taken for the measurements of airway responsiveness, which are terminated once a decline of 15% in FEV1 has been measured. This occurs more often in wheezy subjects.

## Measurements

It is *essential* that in each centre 1000 children have skin prick tests (ISAAC module 3.2) and an examination for flexural dermatitis (ISAAC module 3.1). It is *desirable* that all 1000 children should be tested for bronchial hyper-responsiveness (BHR, ISAAC module 3.3), although if resources are limited, the BHR measurements may be restricted to subgroups as described above.

It is essential that at least 100 wheezy children and 100 non-wheezy children (option B) are tested for bronchial hyperresponsiveness (ISAAC module 3.3), collection of blood for IgE and genetic analyses (ISAAC modules 3.4–3.6) and dust sampled from their homes (ISAAC module 4.1). At the discretion of local investigators, these tests may be extended to a larger subsample, or to the full 1000 children mentioned in option A above.

### Minimum requirements in all 1000 children:

a) Questionnaire to parents enquiring about symptoms of wheezing, rhinitis, eczema (ISAAC modules 1.2–1.4), use of treatments and health services for these complaints (modules 2.2–2.4), additional respiratory symptoms (module 2.1), and risk factors for these diseases (module 2.5).

b) Examination of head, arms and legs for flexural dermatitis (module 3.1).

c) Skin prick tests with allergen extracts of house dust mites (*Der pI* and *Der fI*), cat, *Alternaria tenuis*, mixed grasses pollen, mixed tree pollen, positive (histamine) and negative (diluent) controls. Cockroach and other allergens of local relevance may be added (module 3.2).

**In the subsample of 100 wheezers and 100 non-wheezers:**

d) Measurement of bronchial responsiveness to inhaled hypertonic saline (module 3.3).

e) Blood sampling for total and allergen-specific IgE measurements (to calibrate skin tests, modules 3.4 & 3.5) and storage of dried blood spots for future DNA analysis (module 3.6).

f) Collection and analysis of household dust for aeroallergens (*Der pI*, *Der fI* and *Fel dI*).

## Resource requirements

### Staff

Based on the experience of the German study, a team of one doctor and two nurses can complete tests (b–e) above on 4 children per 1½-hour session. Children were tested in pairs, one pair doing lung function and bronchial challenge tests, while the other pair undergo examination for flexural dermatitis, blood sampling and skin prick tests. The pairs swap over after 40 minutes. Additional periods of 5 minutes at the start and end of the session were allowed for welcome, administration, feedback and dismissal.

If the bronchial responsiveness protocol (d) and blood sampling are omitted, 16 children may be tested per 1½-hour session. To test 200 children with the full protocol (b–e) and 800 with the restricted protocol (b–c), at this rate will take about 100 1½-hour sessions. Spare time after school in the afternoons may be used for home visits by the nurses to collect dust specimens, and for survey administration.

### Equipment and consumables

The main expense is the equipment for measurement of airways responsiveness. Each centre will also require a centrifuge and freezer for processing and storage of blood specimens and a 800W vacuum cleaner suitable for domestic dust collection. Consumables will be required for questionnaires, skin prick testing, blood sampling and dust collection. Laboratory analyses (serum IgE, aeroallergens, other blood or urine tests) should be costed separately.

## Ethical considerations

Evidence of local ethical approval will be required for each participating centre. All elements of the study protocol have passed ethical scrutiny in Germany. Confidentiality of data on individuals will be assured by use of identity numbers on all data recording forms. The principal investigator in each centre will hold the link between names and identity numbers in a form and secure location which satisfies the local requirements for data protection.

## Statistical power

Based on prevalence figures obtained in recent German studies, which are towards the upper end of the comparisons cited below, a sample of 1000 children per centre, including 100 wheezy children per centre, have the specified power to detect at the 5% significance level the following differences:

a) with 90% power, a difference in prevalence of wheezing between any two centres of 6% v 10%.

b) with 80% power, a difference in prevalence of severe wheezing between centres of 1% v 3%.

c) with 80% power, a difference in allergic sensitisation between centres of 15% v 20%.

d) with 80% power, a difference in visible flexural dermatitis between centres of 2.5% v 5%.

e) with 80% power, a difference in the prevalence of "abnormal" airway responsiveness among wheezy children of 20% v 40% (assuming 100 wheezy children per centre).

Option A has 90% power to detect a difference in the prevalence of "abnormal" airway responsiveness to hypertonic saline among all children of 10% v 15%. Option B has 80% power to detect a difference in the weighted prevalence of "abnormal" airway responsiveness among all children of 10% v 20% (assuming prevalences of recent wheeze in the range 8–16%, and prevalences of responsiveness of 30% among wheezers and 9% among other children, similar to findings of the German ISAAC Phase II studies. Details are available from Dr Weiland.

# MODULES

## 1 Core questionnaires

### Module 1.1: Demographic characteristics

In this questionnaire "your child" refers to the child who brought the questionnaire home from school. Please answer the questions by ticking a box or writing in the spaces provided.

1. Is your child a boy or a girl? Boy ❑ Girl ❑

2. When was your child born? _____/_____/_____ Day / Month / Year

3. Was your child born in *xxx*? Yes ❑ No ❑

   If no, in which country? _____

4. In what year was the child's mother born? _____

5. Was she born in *xxx*? Yes ❑ No ❑

   If no, in which country? _____

6. In what year was the child's father born? _____

7. Was he born in *xxx*? Yes ❑ No ❑

   If no, in which country? _____

8. For how long did the child's parents attend school or professional training?

| | Mother | Father |
|---|---|---|
| School | _____ years | _____ years |
| College / University | _____ years | _____ years |

9. Who has answered this questionnaire? Father ❑
Mother ❑
Other person ❑

10. When was the questionnaire answered? _____/_____/_____
Day / Month / Year

**Comments to investigators:**

In **Q 3**, **Q 5** and **Q 7** xxx should be replaced by the country where the study is carried out.
**Q 8:** This question is largely designed for within centre comparisons by socio-economic status and may be modified according to local needs.

## Module 1.2: Questionnaire on wheezing

1. Has your child ever had wheezing or whistling in the chest at any time in the past? — Yes ❑ / No ❑

*IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6.*

---

2. Has your child had wheezing or whistling in the chest in the last 12 months? — Yes ❑ / No ❑

*IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6.*

---

3. How many attacks of wheezing has your child had in the last 12 months?
   - None ❑
   - 1 to 3 ❑
   - 4 to 12 ❑
   - More than 12 ❑

4. In the last 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?
   - Never woken with wheezing ❑
   - Less than one night per week ❑
   - One or more nights per week ❑

5. In the last 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths? — Yes ❑ / No ❑

   [Additional questions about wheezing may be inserted here]

---

6. Has your child ever had asthma? — Yes ❑ / No ❑

7. In the last 12 months, has your child's chest sounded wheezy during or after exercise? — Yes ❑ / No ❑

8. In the last 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection? — Yes ❑ / No ❑

## Module 1.3: Questionnaire on rhinitis

All questions are about problems which occur when your child DOES NOT have a cold or the 'flu.

1. Has your child ever had a problem with sneezing or a runny or blocked nose, when he/she DID NOT have a cold or the 'flu?
   Yes ❑
   No ❑

   *IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6.*

---

2. In the past 12 months, has your child had a problem with sneezing or a runny or blocked nose when he/she DID NOT have a cold or the 'flu?
   Yes ❑
   No ❑

   *IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 6.*

---

3. In the past 12 months, has this nose problem been accompanied by itchy-watery eyes?
   Yes ❑
   No ❑

4. In which of the past 12 months did this nose problem occur?
   *(Please tick any which apply)*

| | | | | | |
|---|---|---|---|---|---|
| January | ❑ | May | ❑ | September | ❑ |
| February | ❑ | June | ❑ | October | ❑ |
| March | ❑ | July | ❑ | November | ❑ |
| April | ❑ | August | ❑ | December | ❑ |

5. In the past 12 months, how much did this nose problem interfere with your child's daily activities?

   Not at all ❑
   A little ❑
   A moderate amount ❑
   A lot ❑

   [Additional questions about rhinitis may be inserted here]

---

6. Has your child ever had hay fever?
   Yes ❑
   No ❑

## Module 1.4: Questionnaire on eczema

1. Has your child ever had an itchy rash which was coming and going for at least six months?
   - Yes ❑
   - No ❑

*IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 7.*

---

2. Has your child had this itchy rash at any time in the last 12 months?
   - Yes ❑
   - No ❑

*IF YOU HAVE ANSWERED "NO" PLEASE SKIP TO QUESTION 7.*

---

3. Has this itchy rash at any time affected any of the following places:
   - Yes ❑
   - No ❑

   the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?

4. At what age did this itchy rash first occur?
   - Under 2 years ❑
   - Age 2-4 years ❑
   - Age 5 or more ❑

5. Has this rash cleared completely at any time during the last 12 months?
   - Yes ❑
   - No ❑

6. In the last 12 months, how often, on average, has your child been kept awake at night by this itchy rash?
   - Never in the last 12 months ❑
   - Less than one night per week ❑
   - One or more nights per week ❑

   [Additional questions about the rash may be inserted here]

---

7. Has your child ever had eczema?
   - Yes ❑
   - No ❑

# 2 Supplementary questionnaires

## Module 2.1: Additional respiratory questions

Although the core questionnaires developed for ISAAC Phase I should prove adequate for between-centre comparisons of the prevalence of wheezing illness, it was considered desirable to develop additional questions in order to:

1. Refine case-definition by distinguishing between symptoms due to asthma and other common respiratory disorders. These may include:
   a) In all countries: acute infections with associated wheeze e.g. bronchitis, bronchiolitis.
   b) In developing countries: suppurative lung disease, tropical pulmonary eosinophilia.
2. Examine the relationship between asthma and other respiratory conditions.
3. Examine the distribution of other respiratory conditions in their own right, particularly where the health effects of ambient air pollution are of concern.

Two groups of questions are proposed: each set may be used separately or in combination. They are presented in a form suitable for parent-completion. The repeatability of these questions has been studied in South Wales, UK. Details are available from Dr Burr (module co-ordinator).

## Cough and phlegm

1. In the last 12 months, has your child usually seemed congested in the chest or coughed up phlegm (mucus) with colds? Yes ❑ No ❑

2. In the last 12 months, has your child usually seemed congested in the chest or coughed up phlegm (mucus) when he/she did not have a cold? Yes ❑ No ❑

*IF YOU HAVE ANSWERED "NO" TO BOTH OF THESE QUESTIONS, PLEASE SKIP QUESTIONS 3 & 4.*

---

3. Does your child seem congested in the chest or cough up phlegm (mucus) on most days (4 or more days a week) for as much as 3 months of the year? Yes ❑ No ❑

*IF YOU HAVE ANSWERED "NO", PLEASE SKIP QUESTION 4.*

---

4. For how many years has this happened? _____ years

---

## Wheeze and breathlessness

1. In the last 12 months, has your child's chest sounded wheezy during or after exercise? Yes ❑ No ❑

2. In the last 12 months, has your child's chest sounded wheezy when he or she had not recently taken exercise? Yes ❑ No ❑

3. In the last 12 months, has your child had wheezing or whistling in the chest when he/she had a cold or the 'flu? Yes ❑ No ❑

4. In the last 12 months, has your child had wheezing or whistling in the chest when he/she did not have a cold or the 'flu? Yes ❑ No ❑

5. Has your child woken up with shortness of breath at any time in his or her life? Yes ❑ No ❑

6. Has your child woken up with tightness of the chest at any time in his or her life? Yes ❑ No ❑

7. In the last 12 months, what has made your child's wheezing worse?

   (Tick all that apply)

   - Weather ❑
   - Pollen ❑
   - Emotion ❑
   - Fumes ❑
   - Dust ❑
   - Pets ❑
   - Wool clothing ❑
   - Colds or 'flu ❑
   - Cigarette smoke ❑
   - Foods or drinks ❑
   - Soaps, sprays or detergents ❑
   - Other things (please list below) ❑

## Questionnaires on disease management (modules 2.2–2.4)

Different patterns of medical care may contribute to variations in the severity of asthma, rhinitis and eczema between countries or over time. Information on the use of medical services is essential to the interpretation of such data as may be routinely available on deaths, hospital admissions and primary care consultations related to these diseases.

The questions focus on the three categories of data: medication, management and health care utilisation. Similar questionnaires have been compiled for each of the three allergic diseases. These may be used separately or in combination, depending upon the purposes of the local study. Possible uses of this data are to:

1. Describe patterns of therapy and management of asthma.
2. Explore the relationship (cross-sectionally) between treatment and morbidity.
3. Compare therapy between countries.
4. Monitor trends in therapy over time.

Unless this module is delivered at the same time as the Phase I questionnaires, it is recommended that the core symptom questions (ISAAC modules 1.2, 1.3 and 1.4) should be administered concurrently. This will permit an analysis of the relationship between treatment and morbidity (point 2 above).

Where the questionnaires refer to "traditional" therapies, the term "traditional" should be changed to suit the cultural context. For example in Australia it would be appropriate to use the term "alternative" therapies.

## Module 2.2: Asthma management

1. In the past 12 months, has your child used any medicines, pills, puffers or other medication for wheezing or asthma? Yes ❑ No ❑

*IF YOU HAVE ANSWERED "YES", THEN PLEASE NAME THE MEDICATION(S):*

| **"Western" medicines** | **How often?** *(please circle one or both)* | |
|---|---|---|
| _____ | When wheezy / regularly | **"regularly"** means every day for at least two months of the year |
| _____ | When wheezy / regularly | |
| _____ | When wheezy / regularly | |
| _____ | When wheezy / regularly | |
| **"Traditional" therapies** | | |
| _____ | When wheezy / regularly | |
| _____ | When wheezy / regularly | |

2. In the past 12 months, has your child used any medicines, pills, puffers or other medication for wheezing or asthma before, during or after exercise? Yes ❑ No ❑

*IF YOU HAVE ANSWERED "YES", THEN PLEASE NAME THE MEDICATION(S):*

**"Western" medicines**

_____

_____

_____

**"Traditional" therapies**

_____

_____

_____

3. Do you have written plan which tells you how to look after your child's asthma? Yes ❑ No ❑

4. Does your child have a peak flow meter at home? Yes ❑ No ❑

5. In the past 12 months, how many visits has your child made to any of the following health professionals for wheezing or asthma?

a) For a wheezy episode?

| | None | 1-3 | 4-12 | More than 12 |
|---|---|---|---|---|
| Health worker | ❑ | ❑ | ❑ | ❑ |
| Nurse | ❑ | ❑ | ❑ | ❑ |
| Doctor | ❑ | ❑ | ❑ | ❑ |
| Hospital emergency department | ❑ | ❑ | ❑ | ❑ |

b) For a regular "check-up" for asthma?

| | None | 1-3 | 4-12 | More than 12 |
|---|---|---|---|---|
| Health worker | ❑ | ❑ | ❑ | ❑ |
| Nurse | ❑ | ❑ | ❑ | ❑ |
| Family doctor | ❑ | ❑ | ❑ | ❑ |
| Specialist | ❑ | ❑ | ❑ | ❑ |
| Hospital emergency department | ❑ | ❑ | ❑ | ❑ |

6. In the past 12 months, how many times has your child been admitted to hospital because of wheezing or asthma?
   None ❑
   1 ❑
   2 ❑
   More than 2 ❑

7. In the past 12 months, has your child been to any of the following for wheezing or asthma?

| | | | | |
|---|---|---|---|---|
| Acupuncturist | Yes | ❑ | No | ❑ |
| Chiropractor | Yes | ❑ | No | ❑ |
| Homeopath | Yes | ❑ | No | ❑ |
| Physiotherapist | Yes | ❑ | No | ❑ |
| Psychiatrist or psychologist | Yes | ❑ | No | ❑ |
| Social worker | Yes | ❑ | No | ❑ |
| Other (please specify) | Yes | ❑ | No | ❑ |

_____

| | | | |
|---|---|---|---|
| 8. | Has your child ever had an allergy injection to prevent or treat asthma? | Yes<br>No | ❑<br>❑ |
| 9. | In the past 12 months, how many days (or part days) of school has your child missed because of wheezing or asthma? | None<br>1 to 5<br>6 to 10<br>More than 10 | ❑<br>❑<br>❑<br>❑ |

## Module 2.3: Rhinitis management

1. In the past 12 months, has your child used any medicines, pills, nose sprays or other medication for hay fever or nose problems? Yes ❑ No ❑

   *IF YOU HAVE ANSWERED "YES", THEN PLEASE NAME THE MEDICATION(S):*

| **"Western" medicines** | **How often?** *(please circle one or both)* | |
|---|---|---|
| _____ | When irritated / regularly | **"regularly"** means every day for at least two months of the year |
| _____ | When irritated / regularly | |
| _____ | When irritated / regularly | |
| **"Traditional" therapies** | | |
| _____ | When irritated / regularly | |
| _____ | When irritated / regularly | |

2. In the past 12 months, how many visits has your child made to a health professional for hay fever or nose problems?

| | None | 1-3 | 4-12 | More than 12 |
|---|---|---|---|---|
| Pharmacist / chemist | ❑ | ❑ | ❑ | ❑ |
| Health worker | ❑ | ❑ | ❑ | ❑ |
| Nurse | ❑ | ❑ | ❑ | ❑ |
| Family doctor | ❑ | ❑ | ❑ | ❑ |
| Specialist | ❑ | ❑ | ❑ | ❑ |
| Hospital emergency department | ❑ | ❑ | ❑ | ❑ |

3. In the past 12 months, has your child had an allergy injection to prevent or treat hay fever or nose problems? Yes ❑ No ❑

4. In the past 12 months, has your child been to a chiropractor, acupuncturist, homeopath or other alternative health care provider for hay fever or nose problems? Yes ❑ No ❑

5. In the past 12 months, how many days (or part days) of school has your child missed because of hay fever or nose problems?
   None ❑
   1 to 5 ❑
   6 to 10 ❑
   More than 10 ❑

## Module 2.4: Eczema management

1. In the past 12 months, has your child used any medicines, ointments, creams, pills or other medications for an itchy skin rash or eczema? Yes ❑ No ❑

*IF YOU HAVE ANSWERED "YES", THEN PLEASE NAME THE MEDICATION(S):*

| **"Western" medicines** | **How often?** *(please circle one or both)* | |
|---|---|---|
| _____ | When itching / regularly | **"regularly"** means every day for at least two months of the year |
| _____ | When itching / regularly | |
| _____ | When itching / regularly | |
| _____ | When itching / regularly | |
| **"Traditional" therapies** | | |
| _____ | When itching / regularly | |
| _____ | When itching / regularly | |
| _____ | When itching / regularly | |

2. In the past 12 months, how many visits has your child made to a health professional for his or her itchy skin rash or eczema?

| | None | 1-3 | 4-12 | More than 12 |
|---|---|---|---|---|
| Pharmacist / chemist | ❑ | ❑ | ❑ | ❑ |
| Health worker | ❑ | ❑ | ❑ | ❑ |
| Nurse | ❑ | ❑ | ❑ | ❑ |
| Family doctor | ❑ | ❑ | ❑ | ❑ |
| Specialist | ❑ | ❑ | ❑ | ❑ |
| Hospital emergency department | ❑ | ❑ | ❑ | ❑ |
| Other (please specify) | ❑ | ❑ | ❑ | ❑ |

_____

| | | | |
|---|---|---|---|
| 3. | <u>In the past 12 months</u>, has your child been admitted to a hospital ward because of an itchy skin rash or eczema? | Yes<br>No | ❑<br>❑ |
| 4. | In the past 12 months, how many days (or part days) of school has your child missed because of an itchy skin rash or eczema? | None<br>1 to 5<br>6 to 10<br>More than 10 | ❑<br>❑<br>❑<br>❑ |

## Module 2.5: Risk factor questionnaire

The purpose of this questionnaire is the standardisation of questions on potential risk factors (and/or confounders) for asthma and allergies in children across the international centres participating in ISAAC Phase II. Inclusion of standardised questions on past and present living and exposure conditions will permit:

a) between-centre (ecological) correlations of disease prevalence and risk factor distribution.
b) a pooled evaluation (meta-analysis) of within-centre analyses of the associations between disease and risk factors at the individual level.

The questionnaire is mainly derived from items which have been used success-fully in the German ISAAC Phase II studies. In Germany, all questions were included in a single questionnaire which also included the core questionnaires. However, it is anticipated that some researchers will wish to reduce the size of the initial questionnaire. In this case, the risk factor questionnaire may be used only in those who agree to the child's participation in the physical examinations.

For many of the questions, locally relevant responses may be added to those suggested here, but it is recommended that no response categories are removed. The following comments for the investigators refer to specific questions:

Comments to investigators:

| | |
|---|---|
| Q 7 and Q 8: | We mean facilities where the children get together with a group of other children. |
| Q 11: | Additional vaccinations of local interest may be added. The questions on the age and the frequency of vaccinations are optional. |
| Q 12: | Additional infections of local interest may be added. The questions on the age of infection are optional. |
| Q 14: | Other pets of local interest may be added. |
| Q 15: | Other animals of local interest may be added. |
| Q 18 and Q 20: | Other fuels of local interest may be added. |
| Q 26: | Other materials may be added if of local interest. |
| Q 28: | The questions on the child's age at the changes are optional. |
| Q 30 and Q 31: | This information may prove very useful for small area analyses. |
| Q 32: | By exercise we mean play or sport activities. |

## Early days

1. How much did your child weigh at birth?

   Less than 1500 g ❑
   1500 to 1999 g ❑
   2000 to 2499 g ❑
   2500 to 3499 g ❑
   More than 3500 g ❑
   Don't know ❑

2. Was your child born within 3 weeks of the calculated date?

   Yes ❑
   No, more than 3 weeks early ❑
   No, more than 3 weeks late ❑
   Don't know ❑

3. Is your child a twin? Yes ❑
   No ❑

4. Was your child ever breast fed? Yes ❑
   No ❑

   If yes, for how long?
   Less than 6 months ❑
   6-12 months ❑
   More than one year ❑

   If yes, for how long was your child breast fed without adding other foods or juices?
   Less than two months ❑
   2-4 months ❑
   5-6 months ❑
   More than 6 months ❑

5. Does your child have any <u>older</u> brothers or sisters?

   No ❑
   Yes ❑ If yes, how many <u>older</u> brothers? _____
   how many <u>older</u> sisters? _____

6. Does your child have any younger brothers or sisters?

No ❑
Yes ❑ If yes, how many younger brothers? _____
how many younger sisters? _____

7. Did your child ever go to a child care facility or nursery school?

No ❑
Yes ❑ If yes, from what age? _____ years

8. Did your child ever go to a kindergarten?

No ❑
Yes ❑ If yes, from what age? _____ years

## Diseases and immunisations

9. Has the child's mother ever had any of the following diseases?
*(tick as many boxes as apply)*

Asthma ❑
Hay fever ❑
Eczema ❑

10. Has the child's father ever had any of the following diseases?
*(tick as many boxes as apply)*

Asthma ❑
Hay fever ❑
Eczema ❑

11. Has your child been vaccinated against any of the following diseases?
*(tick as many boxes as apply)*

| | | | |
|---|---|---|---|
| Pertussis (Whooping cough) (alone or in combination with Diptheria and Tetanus) | Yes | ❑ | If yes, at what age? ___ years |
| | No | ❑ | |
| Measles (alone or in combination with Mumps and Rubella) | Yes | ❑ | If yes, at what age? ___ years |
| | No | ❑ | |
| Tuberculosis/BCG | Yes | ❑ | If yes, at what age? ___ years |
| | No | ❑ | |

12. Has your child ever had any of the following diseases?
*(tick as many boxes as apply)*

| | | | |
|---|---|---|---|
| Measles | Yes | ❑ | If yes, at what age? ___ years |
| | No | ❑ | |
| Whooping cough | Yes | ❑ | If yes, at what age? ___ years |
| | No | ❑ | |
| Tuberculosis | Yes | ❑ | If yes, at what age? ___ years |
| | No | ❑ | |
| Worm infection | Yes | ❑ | If yes, at what age? ___ years |
| | No | ❑ | |

## Your Home

In this section we ask a number of questions on your child's home. For each question, please provide answers for the home in which your child lives at present, and for the home in which your child lived during the first year of life. (In case you have moved, please choose the home in which your child spent most of his or her time during the first year of life). Please make sure that you tick both columns!

13. Does or did your child share the bedroom with other people (adults or children)

| | At present | During the child's first year of life |
|---|---|---|
| Yes | ❑ | ❑ |
| No | ❑ | ❑ |

14. Which of the following pets do or did you keep inside your child's home?

| | At present | During the child's first year of life |
|---|---|---|
| Dog | ❑ | ❑ |
| Cat | ❑ | ❑ |
| Other furry pets | ❑ | ❑ |
| Bird | ❑ | ❑ |
| Others | ❑ | ❑ |

15. Does or did your child have at least once a week contact with any of the following animals outside your child's home?

| | At present | During the child's first year of life |
|---|---|---|
| Dog | ❑ | ❑ |
| Cat | ❑ | ❑ |
| Farm animals | ❑ | ❑ |
| Other animals | ❑ | ❑ |

16. Does or did your child's mother smoke?

| | At present | During the child's first year of life | During pregnancy with your child |
|---|---|---|---|
| Yes | ❑ | ❑ | ❑ |
| No | ❑ | ❑ | ❑ |

17. Does anybody, <u>at present</u>, smoke <u>inside</u> your child's home? Yes ❑ No ❑

If yes, how many cigarettes in total are smoked per day in the child's home? (e.g. mother smokes 4 + father smokes 5 + other persons smoke 3 = 12 cigarettes)

Less than 10 cigarettes ❑
10-20 cigarettes ❑
More than 20 cigarettes ❑

18. Which fuel do or did you use for cooking?
*(tick as many boxes as apply)*

| | At present | During the child's first year of life |
|---|---|---|
| Electricity | ❑ | ❑ |
| Gas | ❑ | ❑ |
| Coal or wood | ❑ | ❑ |
| Other | ❑ | ❑ |

19. How is or was your child's home heated?
*(tick as many boxes as apply)*

| | At present | During the child's first year of life |
|---|---|---|
| One fire, stove or boiler inside the home | ❑ | ❑ |
| More than one fire, stove or Boiler inside the home | ❑ | ❑ |
| A fire, stove or boiler outside the home | ❑ | ❑ |
| Not heated | ❑ | ❑ |

20. Which fuel do or did you use for heating?
*(tick as many boxes as apply)*

| | At present | During the child's first year of life |
|---|---|---|
| Gas | ❑ | ❑ |
| Oil | ❑ | ❑ |
| Electricity | ❑ | ❑ |
| Coal or coke | ❑ | ❑ |
| Wood | ❑ | ❑ |
| Other | ❑ | ❑ |

21. Does or did your child's home have air conditioning?

| | At present | During the child's first year of life |
|---|---|---|
| Yes | ❑ | ❑ |
| No | ❑ | ❑ |

22. Does or did your child's home have damp spots on the walls or ceiling?

| | At present | During the child's first year of life |
|---|---|---|
| Yes | ❑ | ❑ |
| No | ❑ | ❑ |

23. Does or did your child's home have visible moulds or fungus on the walls or ceiling?

| | At present | During the child's first year of life |
|---|---|---|
| Yes | ❑ | ❑ |
| No | ❑ | ❑ |

24. What kind of floor covering is or was there in your child's bedroom?

| | At present | During the child's first year of life |
|---|---|---|
| Fitted carpets | ❑ | ❑ |
| Loose carpets | ❑ | ❑ |
| Bare floor | ❑ | ❑ |

25. What kind of windows are or were there in your child's bedroom? *(tick as many boxes as apply)*

| | At present | During the child's first year of life |
|---|---|---|
| Single glazing | ❑ | ❑ |
| Secondary window | ❑ | ❑ |
| Sealed unit / double glazing | ❑ | ❑ |
| No windows | ❑ | ❑ |

26. What kind of pillow does or did your child use?
*(tick as many boxes as apply)*

| | At present | During the child's first year of life |
|---|---|---|
| Foam | ❑ | ❑ |
| Synthetic fibre | ❑ | ❑ |
| Feather | ❑ | ❑ |
| Other | ❑ | ❑ |
| Does not use a pillow | ❑ | ❑ |

27. What kind of bedding does or did your child use?
*(tick as many boxes as apply)*

| | At present | During the child's first year of life |
|---|---|---|
| Synthetic quilt | ❑ | ❑ |
| Feather quilt | ❑ | ❑ |
| Blankets | ❑ | ❑ |
| Other materials | ❑ | ❑ |

28. Have you made any changes in your home because your child had asthma or allergic problems? *(tick as many boxes as apply)*

| | | | |
|---|---|---|---|
| Removed pets | Yes | ❑ | If yes, at what age of the child? _____ years |
| | No | ❑ | |
| Stopped or reduced smoking | Yes | ❑ | If yes, at what age of the child? _____ years |
| | No | ❑ | |
| Changed pillows | Yes | ❑ | If yes, at what age of the child? _____ years |
| | No | ❑ | |
| Changed bedding | Yes | ❑ | If yes, at what age of the child? _____ years |
| | No | ❑ | |
| Changed floor covering | Yes | ❑ | If yes, at what age of the child? _____ years |
| | No | ❑ | |
| Other changes | Yes | ❑ | If yes, at what age of the child? _____ years<br>If yes, Please describe _____ |
| | No | ❑ | |

29. How would you describe the surroundings of your child's home?

| | At present | During the child's first year of life |
|---|---|---|
| Rural, open spaces or fields nearby | ❑ | ❑ |
| Suburban, with many parks or gardens | ❑ | ❑ |
| Suburban, with few parks or gardens | ❑ | ❑ |
| Urban with no parks or gardens | ❑ | ❑ |

30. What is the name of your child's street of residence?

_____

31. What is the postal code of your child's home?

_____

## Odds and ends

32. Outside school hours, how often does your child usually exercise so much that he/she gets out of breath or sweats?

| | |
|---|---|
| Every day | ❑ |
| 4-6 times a week | ❑ |
| 2-3 times a week | ❑ |
| Once a week | ❑ |
| Once a month | ❑ |
| Less than once a month | ❑ |

33. How often, on average, does your child eat or drink the following, nowadays?

| | Never | Less than once per week | 1-2 times per week | 3-6 times per week | Once per day or more often |
|---|---|---|---|---|---|
| Meat | ❑ | ❑ | ❑ | ❑ | ❑ |
| Fish | ❑ | ❑ | ❑ | ❑ | ❑ |
| Fresh fruits | ❑ | ❑ | ❑ | ❑ | ❑ |
| Raw green vegetables | ❑ | ❑ | ❑ | ❑ | ❑ |
| Cooked green vegetables | ❑ | ❑ | ❑ | ❑ | ❑ |
| Burger | ❑ | ❑ | ❑ | ❑ | ❑ |
| Fruit juice | ❑ | ❑ | ❑ | ❑ | ❑ |
| Fizzy drinks | ❑ | ❑ | ❑ | ❑ | ❑ |

34. Who has answered this questionnaire?

| | |
|---|---|
| Father | ❑ |
| Mother | ❑ |
| Other person | ❑ |

35. When was the questionnaire answered? _____/_____/_____
Day/Month/Year

# 3 Child contact modules

## Module 3.1: Examination for flexural dermatitis

The presence or absence of visible flexural eczema has been found in British studies to be a valid and repeatable measure [1, 2] which increases the specificity of case definition for atopic dermatitis from 94% (based on the ISAAC questionnaire) to over 98% in a hospital population [3] and 97% in a community survey [4]. The sensitivity of case ascertainment is reduced, because physical examination assesses point prevalence rather than period prevalence, and some cases of non-flexural atopic eczema may be excluded. Nevertheless, direct examination of the skin offers a potentially useful tool for standardised comparisons of atopic eczema prevalence between centres, whether used alone as a measure of point prevalence [5], or in conjunction with the UK diagnostic criteria [6]. The latter approach is recommended.

In a community validation study among 700 children aged 3 to 11 in an ethnically mixed south London population [4, 6], a research nurse was able to correctly ascertain this physical sign after training with a standard set of photographic prints [1]. None of the children objected to having their arms, legs, ankles, face and neck examined, and ascertainment of the sign took less than 1 minute per subject. Female examiners should be available in countries where religious beliefs could pose an obstacle to girls being examined in this way.

Seasonal effects are of possible importance in temperate climates. As a minimum requirement, the date of examination should be recorded. A preferable option is to examine seasonal variations directly by examining half of the children in summer and half in winter, or by reassessing a proportion of the sample in two seasons. It should be noted that seasonal effects are not important when 12 month period prevalence is by questionnaire [3].

The photographic protocol [1] included with this manual is self-explanatory. Laminated copies of this protocol, together with detailed instructions on its use in the field and a set of training photographs are available at a small charge from the module co-ordinator, Dr Hywel Williams. A set of photographs for assessing quality control will be included in the training pack. Quality control will be assessed by comparing each observer's assessment of these standard photographs with Dr Williams' assessment of the same photographs. This centralised approach will achieve a degree of standardisation across centres and highlight markedly discrepant observers during field studies.

### Important

The use of the practical manual "So how do I define atopic eczema?" (provided by Dr Hywel Williams) is strongly recommended. Participation in the quality control tests, which are part of this manual, is necessary for inclusion in international comparisons.

### References

1. Williams HC, Forsdyke H, Boodoo G, Hay RJ, Burney PGJ. A protocol for recording the sign of visible flexural dermatitis. *Br J Dermatol* 1995; 133: 941-949.
2. Williams HC, Burney PGJ, Strachan D, Hay RJ. The UK Working Party's diagnostic criteria for atopic dermatitis. II. Observer variation of clinical diagnosis and signs of atopic dermatitis. *Br J Dermatol* 1994; 131: 397-405.
3. Williams HC, Burney PGJ, Pembroke AC, Hay RJ. The UK Working Party's diagnostic criteria for atopic dermatitis. III. Independent hospital validation. *Br J Dermatol* 1994; 131: 406-416.
4. Williams HC, Burney PGJ, Pembroke AC, Hay RJ. Validation of the UK diagnostic criteria for atopic dermatitis in a population setting. *Br J Dermatol* 1996; 135: 12-17.
5. Williams HC, Pembroke AC, Forsdyke H, Boodoo G, Hay RJ, Burney PGJ. London-born black Caribbean children are at increased risk of atopic dermatitis. *J Am Acad Dermatol* 1995; 32: 212-217.
6. Williams HC, Burney PGJ, Hay RJ, et al. The UK Working Party's diagnostic criteria for atopic dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br J Dermatol* 1994; 131: 383-396.

### SKIN EXAMINATION RECORD SHEET

ID number: _____

Date: _____/_____/_____ Field worker number: _____

Has the child signs of visible flexural dermatitis at any of the five following areas?

| | | Yes | No |
|---|---|---|---|
| 1. | Around the eyes | ❑ | ❑ |
| 2. | Around the sides or front of the neck | ❑ | ❑ |
| 3. | Fronts of the elbows | ❑ | ❑ |
| 4. | Behind the knees | ❑ | ❑ |
| 5. | Fronts of the ankles | ❑ | ❑ |

## Module 3.2: Skin prick tests for atopy

### Aims

1. To provide an objective measure of atopy for comparisons within and between centres. "Atopy" may be defined as skin test reactivity to one or more of the following allergens: house dust mites (*Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*); cat fur; mixed grass pollen; mixed tree pollen; and the outdoor mould genus *Alternaria*.
2. To compare the prevalence and degree of sensitivity to individual allergens which are ubiquitous within and between centres (e.g. mites, cat, *Alternaria*).

### Methods

When selecting an appropriate skin prick test method for ISAAC several criteria have been applied: reproducibility under field conditions; simplicity of application; safety; acceptability; quality control; suitability for all ISAAC age groups; low cost; and worldwide availability.

The ALK lancet has been chosen for a number of reasons. Reproducibility and precision with both histamine and allergen extracts has been shown to be good [1]. The application is simple, safe and accepted by children, parents and field workers. There is a good body of knowledge in the medical literature on this skin prick test method and it has already been applied in many surveys. The ALK lancet is available throughout the world.

The "core" allergen extracts to be tested on the *left* forearm are:

| | |
|---|---|
| Histamine 10 mg/ml (positive control) | Diluent (negative control) |
| *D. pteronyssinus* | *D. farinae* |
| Cat | *Alternaria tenuis* |
| Mixed grasses | Mixed trees |

In addition, each centre may add up to eight allergens of their choice by testing them on the *right* forearm. The local relevance of cockroach, artemesia, olive and ragweed should be considered. All of these extracts should be purchased from ALK Denmark. In addition, local allergens that are most prevalent in the respective study area should be included and purchased if possible from ALK, or if not available from ALK, from other companies.

All extracts and the control solutions should be obtained from ALK Laboratories (full address listed on page 65). The allergen extracts are highly standardised and can be delivered throughout the world, including the USA. Histamine 10 mg/ml has been chosen as a positive control solution

because of better reproducibility and precision than alternative positive control solutions [2]. The grass extract is a mixture of commonly occurring grasses in central Europe, i.e.: *Dactylis glomerata*, *Lolium perennae*, *Festuca pratensis*, *Poa pratensis*, *Phleum pratense* and *Avena eliator*. The tree extract is a mixture of commonly occurring tree pollen in central Europe, i.e.: *Betula verrucosa* (birch), *Alnus glutinosa* (alder) and *Corylus avellana* (hazel).

There is a circadian rhythm in the size of skin prick reactions to allergens and histamine [3], so all skin prick tests should be performed in the morning hours (08:00 to 13:00, local time). The site of testing should be free of eczema. An ALK tape with numbers indicating the sequence of allergen extracts is placed in the middle of the volar aspect of the left forearm, 3 cm distal to the elbow crease. One drop of each skin prick solution is placed on the forearm in the above order, on the left and right sides of the tape, respectively. A separate ALK lancet is pricked vertically through each drop with firm pressure. All drops and the tape are removed immediately after the pricks taking care not to contaminate prick points with a different extract.

Reactions to each skin test solution are measured 15 minutes after the pricks. The contours of each wheal are outlined with a fine filter tip pen. The contours are then transferred to the record sheet by means of translucent tape. The size of each wheal is documented as the mean of the longest diameter (a) and the diameter perpendicular to it at its mid-point (b): i.e. (a+b)/2. Measurements of each diameter are made to the nearest millimetre above.

In dark skin wheals can be recognised more easily under strong oblique light and also by palpating the skin. In persons who spend much time outdoors the thickening of the skin may limit the ability to detect skin prick reactions.

### Training

Field workers should be trained before starting the survey, and their precision retested in the middle and at the end of the survey, since the technique of individual fieldworkers may change over time. Reproducibility should be tested as follows at the start of the survey. At least three series of 16 skin prick tests with histamine 10 mg/ml should be performed by each field worker on the volar surface of the forearm of a volunteer until the coefficient of variation (standard deviation as a percent of the mean) of the last series is less than 20%. Half way through the survey and at the end of fieldwork, each field worker should perform two further

series of 16 skin prick tests with histamine 10 mg/ml on the volar forearm of a volunteer. All results should be documented separately for each fieldworker on the "training" record sheets.

## Validation

Because of difficulties in standardizing the performance of different field workers, validation studies using serum IgE measurements (ISAAC module 3.5) are highly recommended in a subsample of children. Where possible, multi-centre comparisons should adopt a cross-over allocation of fieldworkers to the different study areas, so that approximately equal numbers of children are tested by each observer in each centre. Otherwise, it may become impossible to disentangle differences in the performance of different fieldworkers from real differences in the prevalence of skin test reactivity in the comparison areas.

## Safety

Slight physical discomfort may result from the prick and itchiness of the larger wheals. Systemic allergic reactions have not been reported with prick testing despite extensive use in epidemiological surveys. Among over 16,000 adults and children tested in the United States NHANES II survey, six subjects fainted after prick testing, compared to 26 faints after venipuncture [4]. Reviews of deaths occurring from immunotherapy and skin testing in the USA found no fatalities that could be attributed to prick, puncture or scratch testing in the absence of intradermal tests or desensitisation immunotherapy [5, 6]. Systemic allergic reactions occur rarely (0.02%) with *intradermal* skin testing among allergic patients [7] but this technique will not be used in ISAAC.

## References

1. Nelson HS, Rosloniec DM, McCall LI, Ilké D. Comparative performance of five commercial prick skin test devices. *J Allergy Clin Immunol* 1993; 92: 750-756.
2. Illi S, Garcia-Marcos L, Hernando V, Guillen JJ, Liese A, von Mutius E. Reproducibility of skin prick test results in epidemiological studies: a comparison of two devices. *Allergy* 1998; 53: 353-358.
3. Taudorf E, Malling HJ, Laursen LC, Lanner A, Weeke B. Reproducibility of histamine skin prick tests. Inter- and intra-observer variation using histamine dihydrochloride 1,5 and 10 mg/ml. *Allergy* 1985; 40: 344-349.

4. Dreborg S (ed). Skin tests used in type I allergy testing. Position paper prepared by the sub-committee on skin tests of the European Academy of Allergology and Clinical Immunology. *Allergy* 1989; 44[Suppl]: 22-30, 52-59.
5. Turkeltaub PC, Gergen PJ. The risk of adverse reactions from percutaneous prick-puncture allergen skin testing, venipuncture and body measurements: data from the second National Health and Nutrition Examination Survey 1976-1980 (NHANES II). *J Allergy Clin Immunol* 1989; 84: 886-890.
6. Lockey RF, Benedict LM, Turkeltaub PC, Bukantz SC. Fatalities from immunotherapy and skin testing. *J Allergy Clin Immunol* 1987; 79: 660-677.
7. Reid MJ, Lockey RF, Turkeltaub PC, Platts-Mills TA. Survey of fatalities from skin testing and immunotherapy. *J Allergy Clin Immunol* 1993; 92: 6-15.
8. Lin MS, Tanner E, Lynn J, Friday GA (Jr.). Nonfatal systemic allergic reactions induced by skin testing and immunotherapy. *Ann Allergy* 1993; 71: 557-562.

## Contact address

PD Dr med Erika von Mutius
Kinderklinik der Universität im
Dr von Hauner'schen Kinderspital
Lindwurmstraße 4
D-80337 München 2
GERMANY

Tel: (49) 89 5160 2709
Fax: (49) 89 5160 4452
E-mail: erika.von.mutius@kk-i.med.uni-muenchen.de

## Equipment checklist

- ALK allergen extracts
- Tray for allergen bottles
- ALK skin prick lancets
- ALK tape
- Swabs or tissues
- Sharps disposal container
- Felt tip pen (e.g. Edding 1800 profipen 0.5)
- Alarm clock
- Ruler
- Record sheets

# FIELD MANUAL FOR SKIN PRICK TESTING

## Allergen solutions

| Inner side: | Outer side: |
|---|---|
| 1. Positive control (histamine) | 2. Negative control (glycerin) |
| 3. *D. Pteronyssinus* | 4. *D. farinae* |
| 5. Cat | 6. *Alternaria tenuis* |
| 7. Mixed grasses | 8. Mixed trees |

- Perform these eight tests on the left forearm. Use a similar technique on the right forearm to test other allergens of local interest.
- Place the allergens on the tray in the same order as they are put on the forearm.
- Store allergen solutions in a refrigerator between test sessions.

## Applying the solutions

- Check that the skin of the forearm is free of eczema. The test should not be performed on inflamed or broken skin.
- Place the left arm palm upwards on the table in front of the examiner.
- Paste a prenumbered ALK tape onto the left forearm, in the middle and with the "++" mark 2 cm from the elbow.
- Open the packaging of the ALK lancets before doing the test. They should be placed ready to be taken out of the package with one hand.
- Open the bottles with the allergen solutions.
- Put one drop of each allergen on the left or right side of the tape. Do this always in the same sequence. Do not use too much allergen and take care that the different allergens do not run together or run off the arm.
- Put the bottle back to its position on the tray. Do not change the order of the bottles.
- Always start applying allergens on the inner side, working from top (elbow) to bottom. The numbers 1, 3, 5, 7 on the tape mark the distance (1 cm) between the allergens. Apply allergens numbered 2, 4, 6, 8 on the outer side also from top to bottom.
- The drops of solution 1 and 2, 3 and 4, 5 and 6, as well as 7 and 8 are now next to each other, at the same height on the left and right side of the tape, respectively.

### Performing the prick test

- Always use a new ALK lancet for each allergen.
- Prick the ALK lancet for 2 seconds vertically through the drop into the skin using firm pressure.
- Put the used lancets into the disposable container.
- After pricking wipe the allergens off without mixing them. Use a clean swab or tissue and wipe away from the tape towards the outside of the arm.
- Set the alarm clock for 15 minutes.
- Close the allergen bottles with their own coloured caps.

### Reading the reaction

- After 15 minutes outline the contours of the wheal with a thin felt-tip pen (e.g. Edding 1800 profipen 0.5). Do not spread the skin. Hold the pen vertically. Ensure adequate lighting.
- The contour should be drawn at the outside of the wheal. If there is no reaction mark that non-reactive position with a little dot.
- Write "I" on the skin at the top of the inner side and "O" at the top of the outer side, near the "++" mark on the tape.
- Remove the prenumbered tape.
- Paste a transparent tape onto the wheals to transfer the contours.
- Press the tape onto the skin to make sure that the whole contour is transferred to the sticky side.
- Remove the tape from the skin and paste it into the record sheet.

### Measurement of each wheal

- Record measurements in millimetres, rounded to the next higher integer, using a flexible plastic ruler (e.g. Mérieux multitest).
- Always measure the inside of the felt-tip pen contour.
- Identify and measure the longest diameter first.
- Then drop a perpendicular line through the middle of the longest diameter and measure the length of this line.
- Calculate the mean of the two diameters.

## SKIN PRICK TEST RECORD SHEET

ID number: _____ Area number: _____

Date: _____/_____/_____ Field worker number: _____

VOLAR LOWER **LEFT** ARM Fix tape A here Fix tape B here

| Tape A | Tape B |
|---|---|
| 1. +ve control | 2. -ve control |
| 3. *D. Pteronyssinus* | 4. *D. farinae* |
| 5. Cat | 6. *Alternaria tenuis* |
| 7. Mixed grasses | 8. Mixed trees |

DIAMETERS MEASURED TO THE NEAREST WHOLE MILLIMETRE:

1. Positive control

| Max diam (a) | Min diam (b) |
|---|---|
| _____ | _____ |

2. Negative control

| Max diam (a) | Min diam (b) |
|---|---|
| _____ | _____ |

3. *D. pteronyssinus*

| Max diam (a) | Min diam (b) |
|---|---|
| _____ | _____ |

4. *D. farinae*

| Max diam (a) | Min diam (b) |
|---|---|
| _____ | _____ |

5. Cat

| Max diam (a) | Min diam (b) |
|---|---|
| _____ | _____ |

6. *Alternaria tenuis*

| Max diam (a) | Min diam (b) |
|---|---|
| _____ | _____ |

7. Mixed grasses

| Max diam (a) | Min diam (b) |
|---|---|
| _____ | _____ |

8. Mixed trees

| Max diam (a) | Min diam (b) |
|---|---|
| _____ | _____ |

## SKIN PRICK TRAINING RECORD SHEET (1)

Volunteer number: _____ Area number: _____

Date: _____/_____/_____ Field worker number: _____

VOLAR LOWER **LEFT** ARM Fix tape A here Fix tape B here

Tape A Tape B

1. Histamine 2. Histamine

3. Histamine 4. Histamine

5. Histamine 6. Histamine

7. Histamine 8. Histamine

DIAMETERS MEASURED TO THE NEAREST WHOLE MILLIMETRE:

| 1. Histamine | | 2. Histamine | |
|---|---|---|---|
| Max diam (a) | Min diam (b) | Max diam (a) | Min diam (b) |
| _____ | _____ | _____ | _____ |
| 3. Histamine | | 4. Histamine | |
| Max diam (a) | Min diam (b) | Max diam (a) | Min diam (b) |
| _____ | _____ | _____ | _____ |
| 5. Histamine | | 6. Histamine | |
| Max diam (a) | Min diam (b) | Max diam (a) | Min diam (b) |
| _____ | _____ | _____ | _____ |
| 7. Histamine | | 8. Histamine | |
| Max diam (a) | Min diam (b) | Max diam (a) | Min diam (b) |
| _____ | _____ | _____ | _____ |

Coefficient of variation: _____ %

## SKIN PRICK TRAINING RECORD SHEET (2)

Volunteer number: _____ Area number: _____

Date: _____/_____/_____ Field worker number: _____

VOLAR LOWER **RIGHT** ARM Fix tape A here Fix tape B here

Tape A Tape B

1. Histamine 2. Histamine

3. Histamine 4. Histamine

5. Histamine 6. Histamine

7. Histamine 8. Histamine

DIAMETERS MEASURED TO THE NEAREST WHOLE MILLIMETRE:

| 1. Histamine | | 2. Histamine | |
|---|---|---|---|
| Max diam (a) | Min diam (b) | Max diam (a) | Min diam (b) |
| _____ | _____ | _____ | _____ |
| 3. Histamine | | 4. Histamine | |
| Max diam (a) | Min diam (b) | Max diam (a) | Min diam (b) |
| _____ | _____ | _____ | _____ |
| 5. Histamine | | 6. Histamine | |
| Max diam (a) | Min diam (b) | Max diam (a) | Min diam (b) |
| _____ | _____ | _____ | _____ |
| 7. Histamine | | 8. Histamine | |
| Max diam (a) | Min diam (b) | Max diam (a) | Min diam (b) |
| _____ | _____ | _____ | _____ |

Coefficient of variation: _____ %

## Module 3.3: Bronchial responsiveness to hypertonic saline

In many epidemiological surveys of asthma in children, bronchial responsiveness to inhalation of pharmacological agents such as methacholine or histamine has been used as an objective measure of asthma. As these agents become less available in some countries, there has been increasing interest in the use of non-pharmacological agents. Bronchial provocation tests using hyperosmolar aerosols are practical to use in the identification and assessment of the bronchial responsiveness associated with moderate to severe asthma [1]. Challenges with hyperosmolar aerosols require little patient co-operation, the equipment required is portable, relatively cheap, and readily available. Bronchial challenge by inhaling aerosols of hyperosmolar saline has obvious appeal from an ethical standpoint and provides an attractive and cheap alternative to pharmacological agents, allergens or sensitising agents. There may be an advantage in using hyperosmolar challenges in that they provoke airway narrowing indirectly by causing the endogenous release of mediators to which the subject is sensitive. In epidemiological studies, the sensitivity and specificity of challenge with hyperosmolar saline appear to be similar to challenges with methacholine and histamine [2, 3]. In the field these challenges have a sensitivity for current asthma in the order of 50% and a specificity in the order of 90% [3]. The repeatability of the test is good with the relative difference for PD20 and PD15, over a two week period, being a factor of 1.64 for adults [4] and 1.70 for children respectively [5].

### Safety

As with any other bronchial provocation tests, challenge using non-isotonic aerosols leads to airway narrowing that can be of sudden onset and cause a marked reduction in arterial oxygen tension. We recommend the precautions set out in detail by Sterk et al [6] be taken. In brief, we do not recommend a challenge with hyperosmolar saline in children with severe airflow limitation or those with an FEV1 less than 75% predicted. Any child with a medical condition that may be affected adversely by a fall in arterial oxygen should also be excluded from testing. The usual equipment required to reverse an acute attack of asthma (bronchodilators and oxygen) should be at hand. Trained personnel only should administer these challenges and immediate access to medical help should be available. Children should never be left unattended and their airway narrowing

should be reversed to within 90% of baseline before they are allowed to leave. The initial challenge period should only be 30 seconds.

### Medications

Because some medications used in the treatment of asthma can inhibit the response to hyperosmolar saline, subjects should withhold the following asthma medication prior to the challenge test: anti-histamines – 48 hr; theophylline and sustained release bronchodilators – 12 hr; long acting beta2 adrenoceptor agonists – 24 hr; aerosol short acting beta2 adrenoceptor agonists, sodium cromoglycate and nedocromil sodium, – 6 hr. Theoretically, the acute administration of inhaled steroids would have a minimal effect on the response to challenge with hyperosmolar saline. However, regular use of inhaled steroids reduces the sensitivity to hyperosmolar saline and the airway response can be totally inhibited in some people. Regular use of inhaled steroids should be recorded, and any oral steroid therapy taken in the last 2 weeks should be documented.

### Technical considerations

Ultrasonic nebulisers are used to generate non-isotonic aerosols because they produce dense aerosols. Compared to jet nebulisers, ultrasonic nebulisers deliver between 2 and 8 times more aerosol over the same period of time. The manufacturer's specification will usually include the droplet size and distribution. However, this can vary with the viscosity and vapour pressure of the nebuliser fluid, the flow rates generated, and the nature of the apparatus connecting the patient to the nebuliser. The bore and length of the tubing attached to the reservoir is important and will reduce or increase the amount of aerosol delivered to the mouthpiece. The size of the valve may also affect output and should be standardised.

For these reasons we recommend that the output of the nebuliser is measured, by weighing the canister and tubing prior to and on completion of each challenge test, to ensure that the output is adequate for each challenge. When weighing the tubing, it is necessary to cork the end of the tubing to prevent fluid loss. An alternative is to put the end of the tubing onto the other port of the canister while it is being weighed.

There have been some reports of a reduced output of aerosol from the DeVilbiss 99 and 2000 nebulisers. Factors that ensure optimal output include:

1) Keeping electrical terminals free of salt by cleaning regularly with an alcohol swab.

2) Warming solutions to 20–25°C before loading the canister (not straight from the refrigerator).
3) Ensuring that the length of tubing from the nebuliser to the valve is 60–70 cm and not necessarily as supplied with the nebuliser.
4) Ensuring the valve size is adequate so that volume is not lost by excessive condensation.
5) Using the main canister of the nebuliser and **not** the plastic cup insert.
6) Ensuring that the crystal is still able to function properly: it should be checked regularly and replaced if necessary.
7) Ensuring that there is no valve flutter during inspiration.

### Recommended equipment

**Nebuliser**: It is important to ensure that this is of the ultrasonic type and that it delivers an adequate volume of aerosol in an appropriate particle size distribution. A suitable ultrasonic nebuliser is one with a canister that has a volume in excess of 200 ml, that can be easily detached and weighed. The output of the nebuliser should be sufficient to deliver at least 1.5 ml per minute with the tubing intended for use in the study attached. The nebuliser should be capable of generating an aerosol with a mass median diameter of particles between 2 to 5 microns. If there is a facility available to check this for older nebulisers this should be done. We have found that coughing frequency increases with the density of the aerosol and for this reason the output may be adjusted for some subjects, but not below 1.5 ml/min, otherwise the challenge will be inadequate. Coughing as a result of inhaling hyperosmolar saline could affect the volume of the aerosol inhaled and deposited. The cough is usually transient, lasting only a few minutes, and more subjects can complete the challenge without reduction in output of the nebuliser. While the volume of the output for each nebuliser and circuit should be measured for each test, it is acceptable to rely on the manufacturers specifications for particle size distribution. The currently available ultrasonic nebulisers that fulfil the above criteria include **Timeter Compuneb 500** or **DeVilbiss Ultraneb 2000**.

**Tubing**: This should have a smooth interior surface with an internal bore size of 22 mm. The length of the tubing will affect the output of the nebuliser and should be kept constant within a centre. Suggested tubing: **Bennetts Cat No TV 2723** or **DeVilbiss No 8885, Silicon**. Suggested length: approximately 60–70 cm. Please note that the tubing that comes

with a nebuliser may be much longer than this and thus the output would be lower as some of the particles will condense on the wall of the tubing.

**Two-way valve**: The recommended valve are **Hans Rudolph two-way non-rebreathing valve 2700** or **Laerdal valve No 560 200 / 850 500** (ordered through DeVilbiss, manufactured by Dahlhausen, Cologne, Germany). The valves and rubber diaphragms are robust and can be used up to 100 times without replacement. The output and particle size distribution of the aerosol appear to be suitable when this valve is used in conjunction with the recommended nebuliser.

**Mouthpiece**: The valve should be connected to a mouthpiece for inhalation as opposed to a face mask. (e.g. DeVilbiss cut off and used as adapter to mouthpiece for children from Jäger No 892102).

**Spirometer**: This should be portable and comply with the recommendations of the American Thoracic Society Statement on Standardization of Spirometry [7]. It is essential that the variability of the measurements is within appropriate limits to allow accurate measurement of change in lung function. When using a computerised spirometer, the results for each test must be available within 30 seconds to cope with the frequency of measurements required by the protocol.

**Balance**: This must be able to measure the combined weight of the nebuliser canister and tubing yet maintain precision to determine output. An appropriate balance would have a weighing range of 0 to 2500 g and be capable of being read to 0.1 g. (e.g. **Sartorius Basic 2100** or **Mettler 3000**).

Solution: A single concentration of **4.5% saline** (close to sea water) is used. This solution can be prepared by adding 45 g of dialysis grade sodium chloride BP to 1,000 ml of sterile pyrogen-free water. This solution is kept in the refrigerator for 1 week only before being discarded. It should be warmed to room temperature (20–25°C) before loading the canister.

## Procedure

Height and weight are measured without shoes and the predicted $FEV_1$ determined from local reference standards. The subject is then instructed in the forced expiratory manoeuvre and a minimum of two baseline spirograms are recorded. The American Thoracic Society criteria for completion of a satisfactory set of spirograms should be followed. If the first two baseline $FEV_1$ readings are not within 5% of one another, a third spirogram should be performed. The highest of two reproducible (within 5%) measures of $FEV_1$ is

recorded as the baseline $FEV_1$. If this is less than 75% of predicted, no saline is given, an inhaled bronchodilator (e.g. 400 μg salbutamol via PMDI and spacer) is administered, followed by repeat spirometry 10 minutes later, the second $FEV_1$ also being recorded on the form.

The time of inhalation of the saline aerosol is progressively increased. The initial exposure time to the aerosol is 30 secs. One minute later two or three measurements of $FEV_1$ are made. The next challenge period should follow within three minutes of the end of the previous one. If the $FEV_1$ falls less than 10%, the exposure time is doubled. If the fall in $FEV_1$ is between 10 and 15%, the exposure time should be repeated. Should after two repetitions of the exposure time the $FEV_1$ still be 10 to 15% below the baseline value, the duration of the inhalation period should be doubled again according to the protocol. If the fall in $FEV_1$ is greater than 15% the bronchial challenge is stopped. The inhalation periods are 30 secs, 1 min, 2 min, 4 min and 8 min, when repetitions are not needed. In any case the inhalation of hypertonic saline is stopped after a maximum inhalation period of 15.5 minutes. Thus, if repetitions are necessary the duration of the last period must be adopted accordingly.

The canister and tubing to the valve, but not the valve, are weighed before the first and after the final challenge period in order to measure the total dose of aerosol delivered. To obtain the rate of nebulizer output in ml per minute, the total output is divided by the total time of exposure. For details see the record form.

After each subject has completed the study, the mouthpiece and valve should be washed in warm soapy water then rinsed in clean water and dried.

### Expression of the response

Firstly, the dose delivered in each challenge period is calculated by multiplying the output in ml per min by the time of the challenge period. A dose-response curve is constructed by plotting the $FEV_1$ (in litres) or the % change in $FEV_1$, against the cumulative dose of aerosol delivered, expressed in ml on a log scale for each inhalation period.

A value for $PD_{15}$ can be obtained by linear interpolation of the last two points. A horizontal line is drawn from the y-axis at the level representing 85% of the baseline value of $FEV_1$, or if % fall is used, 15%. A vertical line is drawn from the point where this line intersects with the interpolation line

to the x-axis. A computer programme is available from Dr Robertson (module co-ordinator) to facilitate this calculation. Within the asthmatic population the $PD_{15}$ is log normally distributed. For statistical analysis, the values for $PD_{15}$ are compared after log transformation. There are several possible approaches to calculating the results. It is therefore important that all the data recorded below is entered into a database to allow for subsequent analysis using different techniques.

An illustrative example:

| Saline inhalation time | Dose per period (ml) | Cumulative dose (ml) | $FEV_1$ (1st) | $FEV_1$ (2nd) | % fall in $FEV_1$ |
|---|---|---|---|---|---|
| 30 sec | 1.2 | 1.2 | 3.2 | 3.1 | 0 |
| 1.0 min | 2.3 | 3.5 | 3.1 | 3.0 | 3.1 |
| 2.0 min | 4.6 | 8.0 | 2.9 | 2.9 | 9.4 |
| 4.0 min | 9.2 | 17.3 | 2.6 | 2.4 | 18.7 |
| 8.0 min | (test terminated) | | | | |

## Ethical approval

The hypertonic saline challenge has been performed widely in the laboratory and in epidemiological studies in the field, and has been found to be safe and well tolerated [3]. The degree of bronchoconstriction expected would not result in clinically apparent symptoms and can be readily reversed with the inhalation of a bronchodilator. The challenge is designed in dose-response fashion so that only a very small dose is delivered initially, ensuring a safe challenge even for those subjects with very sensitive airways.

A consent form should be signed by the parent or guardian and accompanied by an information sheet explaining the procedure. A suggested paragraph for inclusion in the information sheet follows:

"The hypertonic saline inhalation test is commonly used in respiratory laboratories to detect asthma in children and adults and does not involve the inhalation of chemicals or drugs. Your child will be asked to inhale a fine mist of concentrated saline solution (similar to sea water). The test will be supervised by a doctor from ..... During the test, breathing tests will be carried out to monitor any change. The test is positive when there is a small reduction in the breathing test. The fall is not great enough to cause symptoms and returns to normal within a few minutes following completion of the test."

## References

1. Schoeffel RE, Anderson SD, Altounyan RE. Bronchial hyperreactivity in response to inhalation of ultrasonically nebulised solutions of distilled water and saline. *BMJ* 1981; 283: 1285-1287.
2. Salome CM, Peat JK, Britton WJ, Woolcock AJ. Bronchial hyperresponsiveness in two populations of Australian schoolchildren. I. Relation to respiratory symptoms and diagnosed asthma. *Clin Allergy* 1987; 17: 271-281.
3. Riedler J, Reade T, Dalton M, Holst DI, Robertson CF. Hypertonic saline challenge in an epidemiological survey of asthma in children. *Am J Respir Crit Care Med* 1994; 150: 1632-1639.
4. Anderson SD, Smith CM. The use of non-isotonic aerosols for evaluating bronchial hyperresponsiveness. In: Provocative Challenge Procedures. Spector S, (ed). Futura, Mount Kisco, New York, 1989, p227-252.
5. Riedler J, Reade T, Robertson CF. Repeatability of the response to 4.5% NaCl challenge in children with mild to severe asthma. *Pediatr Pulmonol* 1994; 18: 330-336.
6. Sterk PJ, Fabbri LM, Quanjer PhH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo J-L. Airway responsiveness. Standardized challenge testing with pharmacological, physiological and sensitizing stimuli in man. *Eur Respir J* 1993; 6[Suppl 16]: 53-83.
7. American Thoracic Society. Standardization of spirometry. 1987 update. *Am Rev Respir Dis* 1987; 136: 1285-1289.

A reprint of a detailed review of hypertonic saline challenge is available from Dr Robertson:

Anderson SD, Smith CM, Rodwell LT, du Toit JI, Riedler J, Robertson CF. *The use of nonisotonic aerosols for evaluating bronchial responsiveness*. In: Spector S. (ed) *Provocation testing in clinical practice*. New York: Marcel Dekker, 1995.

## HYPERTONIC SALINE CHALLENGE – EQUIPMENT

With three technicians and two sets of equipment (as below), 25 children can be tested per school day. Two technicians perform the challenge tests while the third organises the subjects and obtains basic data such as height, weight and medications.

- 2 Ultrasonic nebuliser, canister and tubing. Corks optional
- Saline 4.5% – 1000 mls for 25 tests
- 2 Two-way non-rebreathing valves
- 10 Mouthpieces for nebuliser
- 2 Spirometers
- 2 Stopwatches
- 10 Nose clips
- 1 Balance
- 30 Record forms
- 2 Field manuals
- 1 Height measure
- 1 Weighing scales
- 1 Oxygen cylinder, regulator, tubing, nebuliser and face mask
- 1 Bronchodilator – PMDI with spacer and nebulising solution

## HYPERTONIC SALINE CHALLENGE – FIELD MANUAL

### Criteria for Inclusion

- Able to perform baseline spirometry satisfactorily
- $FEV_1 \geq 75\%$ predicted
- No cromoglycate, nedocromil, short-acting bronchodilator or ipratropium bromide for 6 hours prior to test
- No theophyllines for 12 hours prior to test
- No long acting-bronchodilator for 24 hours prior to test
- No antihistamines for 48 hours prior to test

### Nebuliser Set-up

- Ultrasonic nebuliser (output 1.5–2 ml/min, suitable particle size).
- Remove the butterfly valve on the elbow and use the main canister, not the plastic cup insert.
- Fill the ultrasonic nebuliser canister with 4.5% saline (warmed to room temperature) up to the 200 ml mark, and refill after each nebulisation to maintain a volume of more than 150 ml.
- Weigh the filled canister with aerosol tubing and cork (corks are used to seal outlets on tubing and canister).
- Connect the canister to the nebuliser by air tube and transducer cable.
- Set the output knob appropriately (close to maximum setting).
- Attach the inspiratory port of the 2-way valve to the tubing that delivers the aerosol.
- Do not clean the nebuliser with detergent: no greasy substances are used. Clean the terminals of the nebuliser regularly with alcohol swabs.

## Baseline Spirometry

- The subject's height and weight without shoes are measured.
- Baseline $FEV_1$ is recorded twice. The best of these two readings should be used. However, if readings are not within 5% of each other, a further $FEV_1$ manoeuvre should be performed, and the best of these recorded.
- If the $FEV_1$ is < 75% of predicted, an inhaled bronchodilator should be given and $FEV_1$ recorded again after 10 min. An increase in $FEV_1$ of 15% or more will be indicative of a positive bronchodilator response.
- The values for a 10% and 15% fall in $FEV_1$ from baseline are calculated.

## Saline Challenge

- The child should be seated in a comfortable position and encouraged to maintain good posture to enable effective administration of saline.
- The child should be encouraged to breathe normally through a 2-way non-rebreathing valve with a nose clip worn. The child's breathing pattern should be closely observed to ensure that they maintain tidal breathing, not hyperventilation.
  The initial exposure time to the aerosol is 30 seconds. 60 seconds after completion of this inhalation step, two consecutive $FEV_1$ readings are recorded and the highest of these chosen.
- Provided that the $FEV_1$ does not fall by more than 10% the exposure times to the aerosol are then doubled, i.e. 1 minute, 2 minute, 4 minute, and 8 minute time intervals, and spirometry performed 60 seconds after each of these intervals of the challenge with the aerosol.
- The next inhalation period should follow within 3 minutes of completion of the previous one.
- If after any inhalation step, the $FEV_1$ falls to between 10 and 15% of the base-line reading, then the subsequent inhalation period is not doubled, but the previous period is repeated to avoid a dangerous fall in $FEV_1$. Should after two repetitions of the exposure time the $FEV_1$ still be 10 to 15% below the baseline value, the duration of the inhalation period should be doubled again according to the protocol.
- If there is a fall in $FEV_1$ of 15% or more, bronchodilator should be administered and spirometry repeated after 10 minutes.

- The test is terminated after there is a fall in $FEV_1$ of more than 15% or after the total time of exposure is 15.5 minutes (usually after the 8 minute interval). When inhalation periods had to be repeated the duration of the last period must be shortened accordingly.
- The nebuliser chamber plus aerosol tube and corks are weighed after the final challenge step, so that the total amount of nebulised saline can be calculated (amount nebulised = the difference in weight prior and post).
- Children should never be left unattended and their airway narrowing should be reversed to 90% or more of their baseline $FEV_1$ before they are allowed to leave.

### Measuring dose-response

- The amount of aerosol delivered per minute is calculated by dividing the total amount delivered by the time of delivery e.g. 28 ml in 15.5 minutes = 1.81 ml per minute. The dose is expressed cumulatively with time.
- An individual dose-response curve is constructed by plotting the $FEV_1$ in litres on a linear scale against the cumulative dose of aerosol delivered (ml) on a logarithmic scale. $PD_{15}$ $FEV_1$ is obtained by linear interpolation.
- In the children in whom the fall in $FEV_1$ did not reach 15% the fall in $FEV_1$ recorded after the final dose of aerosol and the dose of aerosol delivered are reported.

## 4.5% SALINE CHALLENGE – RECORD SHEET

ID number: _____ Name: _____

Date: _____/_____/_____ Time: _____

Sex: _____ D.O.B.: _____/_____/_____

Height (cm): _____ Weight (kg): _____

Predicted $FEV_1$ (ml): _____ Source of predicted values: _____

Current medications: _____

Last medication: _____ Time taken: _____

Pre-challenge $FEV_1$ (ml): _____ % predicted: _____ % variability: _____

Calculated 10% fall in $FEV_1$: _____ Calculated 15% fall in $FEV_1$: _____

*If $FEV_1$ <65% predicted*: *$FEV_1$ post ß-agonist* (ml): _____ *% increase*: _____

| Saline inhalation time | Dose per period (ml) | Cumulative dose (ml) | $FEV_1$ (1st) | $FEV_1$ (2nd) | % fall in $FEV_1$ |
|---|---|---|---|---|---|
| 30 sec | | | | | |
| 1.0 min | | | | | |
| 2.0 min | | | | | |
| 4.0 min | | | | | |
| 8.0 min | | | | | |

Total inhalation time: _____ min _____ sec

Weight of canister plus tubing — Before challenge: _____ grams

After challenge: _____ grams

Amount nebulised: _____ g (ml)

Output of nebuliser: _____

PD15: _____ ml

## Module 3.4: Blood sampling and frozen storage

Blood samples are collected by venipuncture into plain sample tubes and allowed to clot at room temperature. The serum is separated by centrifugation at 2500-3000 rpm for 15 minutes. This may be done in a laboratory or with a portable centrifuge immediately after collecting the blood. This decision will depend upon whether blood samples are to be analysed for more labile components (e.g. eosinophilic cationic protein).

Serum should be pipetted off and aliquots stored in 1 ml plastic tubes with screw corks (e.g. NUNC, Denmark and USA). The use of tubes with snap closures is strongly discouraged, since these may allow samples to dry out. Each tube should be labelled with an identification number and date of sampling and stored in matching partitioned plastic or cardboard boxes. Each sample should also be documented in a record sheet with the identification number, date and exact time of sampling, exact times of centrifuging and freezing, and the location by box and position within the box.

Samples should be frozen with dry ice in the field and then kept frozen, preferably at -70°C. If this is not feasible, then -20°C storage is sufficient for most, but not all analyses. Specimens should be kept frozen during transit by shipment in dry ice. Repeated freezing and thawing should be avoided as this may limit the use of the specimens for certain assays. For these reasons, each sample should preferably be frozen as multiple aliquots in separate boxes and storage conditions, including freeze-thaw cycles, should be recorded.

The minimum volumes required for analysis are 100 µl serum for total IgE (duplicate assay) plus 50 µl per allergen for specific IgE. For several other analysis, including other immunoglobulin isotypes and cytokines, 25 µl may be adequate.

### Suppliers of specimen tubes

NUNC Inc.
2000 North Aurora Td.
Naperville
IL 60563 – 17969
U.S.A.

NUNC
Box 280
Kamstrup
DK-4000 Roskilde
DENMARK

## Module 3.5: Serum IgE

Elevated serum immunoglobulin E (IgE) levels and the presence of IgE antibodies against specific allergens has been shown to be closely linked to asthma and bronchial hyperresponsiveness in children in western countries, possibly through genetic mechanisms. Even results of well standardised skin prick tests may be subject to bias arising from different field workers and variations in the degree of skin reactivity in different racial groups or under different environ-mental conditions. Moreover, in some countries the most prevalent sensitisation to tree and grass pollen is unknown.

Measurements of total serum IgE may thus provide additional information on:

1. The degree of atopic susceptibility of populations in different centres, although in many, particularly non-industrialised countries total IgE may also be raised in association with parasitic infections.
2. The atopic status of individuals within a population.
3. Validation of comparisons of skin prick responses between centres. Measurement of specific IgE against the allergens tested by the skin prick technique may be particularly informative in this regard.

The assay is performed by Pharmacia Diagnostics which runs a worldwide network of reference laboratories (located in Europe, USA, Canada, Latin America, Australia, Asia and Africa). Further information is available from:

Dr Staffan Ahlstadt
Pharmacia Diagnostics AB
S-75182 Uppsala
SWEDEN

Tel: (46) 18 16 38 03
Fax: (46) 18 14 03 58

## Module 3.6: Storage of dried blood spots for genetic analysis

During the next 10 years it is likely that definite genetic markers for atopy and asthma will be found. Analysis of ISAAC Phase II results will be enhanced by the ability to look for known genetic markers in specimens from the subjects studied and to relate these to other findings. This would require storage of DNA-containing material, such as blood drawn primarily for other reasons.

Two alternative methods are currently available to store DNA from blood samples: immediate buffy coat or DNA extraction with frozen storage; or storage of whole blood as dried spots. Dried blood spots have been used for neonatal screening for a number of diseases. DNA can be successfully identified using polymerase chain reaction (PCR) techniques from dried blood spots at least 10 years old (Dr Andrew Fellowes, Christchurch Molecular Pathology Laboratories, New Zealand; Prof Bob Elliott, University of Auckland, New Zealand, personal communications). There have been no problems with the amplification of DNA using PCR [1-3], with one published case of successful DNA extraction from a dried blood spot 17 years old [4]. However, the DNA extracted from a dried blood spot may be too degraded to use for other methods such as Southern blotting (Dr Andrew Fellowes, personal communication).

### Materials

Use high quality blood test paper such as newborn screening cards (Schliecher and Schuell #903) or Guthrie cards, cost approximately US $0.20 each. This sample collection paper is similar to blotting paper but has special qualities that render it suitable for use in collecting blood spots.

### Technique

- Do not touch the filter paper by hands, gloves, formulas, antiseptic solutions, lotions or other materials at any stage of the collection process in the area where the circles are marked.
- Complete the required information on the card using a ballpoint pen.
- Drop blood onto the circled area of the test paper from a syringe. Completely fill all the circles with blood. Do not layer successive drops of blood more than once in the same collection circle.
- Avoid touching or smearing the blood spots.
- Allow the blood specimens to air dry for at least 3 hours in a horizontal and preferably elevated position (to allow drying from both sides). The

process may be speeded up using a cold hairdryer. The dried blood on the card should look brown, with no trace of redness.

- Do not let the specimen come into contact with any surfaces, direct heat or sunlight. Do not refrigerate the samples.
- When the samples are dry, place each into a separate envelope, or stack with each specimen rotated 180° above the last, to avoid superimposing collection areas.
- The mass of cards may be stored dry and at room temperature.

## Ethics

Emphasis is placed on the ethics of storing blood for future genetic analysis. After obtaining appropriate ethical approval to proceed, an explicit written information letter should be given to each parent or guardian before blood is taken from the child. Informed written consent should be obtained and the consent forms must be kept for as long as the samples are stored. It is important to emphasise that these samples are research samples only and not for diagnostic or health service use. The information letter to the parent or guardian should include the following points:

1. The blood will be stored for use only in future research into asthma and allergies.
2. The blood will not be used for commercial purposes.
3. Parents can specify the limits to which the blood may be used, if they wish.
4. The research data collected will not be given to any other person. The data will be retained in locked filing cabinets and accessed only by authorised personnel.
5. Confidentiality will be maintained at all times.

## References

1. McIntosh I, Strain L, Brock DJH. Prenatal diagnosis of cystic fibrosis where a single affected child has died: Guthrie spots and microvillar enzyme testing. *Lancet* 1988; 2(8619): 1085.
2. Lyonnet S, Caillaud C, Rey F, Berthelon M, Frezal J, Rey J, Munnich A. Guthrie cards for detection of point mutations in phenylketonuria. *Lancet* 1988; 2(8609) :507.
3. *PCR methods and applications*. Review 1991; 99-106. Cold Spring Harbour Laboratory Press. ISSN 1054-9803/91.
4. Williams C, Weber L, Williamson R, Hjelm M. Guthrie spots for DNA-based carrier testing in cystic fibrosis. *Lancet* 1988; 2(8612): 693.

# 4 Environmental module

## Module 4.1: Sampling of dust for determination of allergen content

Indoor allergens are a leading cause of asthma and other allergic diseases [1, 2]. Two international workshops and numerous studies have focused on the importance of the determination of allergen content in dust from homes, day care centres and schools [2]. Based on these publications, the following protocol is recommended for the collection of dust and analysis of allergens.

### Equipment

Vacuum cleaners with at least an 800W engine should be used. The vacuum cleaner should have a protective device to prevent overheating during the sampling procedure.

The ALK filter is suitable for dust collection. This method is more suitable than collecting dust using a paper bag. The filter retains 74% of particles 0.3-0.5 μm, 81% of particles 0.5-1.0 μm, 95% of particles 1-10 μm and virtually 100% of larger particles [3].

### Site and time of collection

Allergen levels have been analysed in dust from many sites indoors. Carpets and upholstered surfaces are the most important reservoirs [2]. The levels may vary considerably in different locations. Dust should be sampled from two sites within homes, e.g. the mattress or sleeping place and either the carpets, upholstery or floor in the living room. The bed should be vacuumed on the sheets or, if there are no sheets, directly on the mattress or sleeping surface. Soft surfaces (beds, carpets, upholstery) are vacuum cleaned for 2 minutes per square metre, covering an area of at least 2 $m^2$ (4 $m^2$ if possible). Hard surfaces are cleaned for 1 min/$m^2$, covering an area of at least 4 $m^2$.

Dust allergen levels are usually higher during the winter in temperate climates [4]. Therefore, dust collection in these climatic zones should preferably be performed between October and January in the northern hemisphere and between April and July in the southern hemisphere.

## Procedure

Sampling should be performed at a defined time, preferably at least three days after the last cleaning, to allow enough material to settle. The time elapsed since the last vacuum cleaning in each room or site sampled should be recorded. After collection of dust, the vacuum cleaner is turned off with the mouth of the filter holder facing up. The filter box with filter is removed, the lid attached and then stored in a plastic bag at -20°C until analysis. After freezing for 2 days (to kill mites) it may be transported at +4°C to +8°C. Each filter box should be clearly marked with the subject's identification number, collection site, date, time and the duration and area of sampling. The filter holder can be cleaned with soap and water. It should be completely dry before it is reused.

## Extraction and processing of dust

Dust samples collected from floors must be sieved through a 300 mm mesh to remove all larger particles which influence the weight of the dust. Sieving is not usually necessary for samples collected from beds, but any large particles should be removed manually before extraction.

Allergen levels may be analysed either using radioimmunoassay or enzyme-linked immunosorbent assay [5, 6]. ELISA is preferable to RIA as it is easier to perform and does not involve use of radioactive materials. Results should be expressed both as the concentration of allergen per gram of fine dust, and as the amount of allergen per square metre of sampling area.

## References

1. Platts-Mills TAE, Ward GW (Jr), Sporik R, Gleber LE, Chapman MD, Heyman PW. Epidemiology of the relationship between exposure to indoor allergens and asthma. *Int Arch Allergy Appl Immunol* 1991; 94: 339-345.
2. Platts-Mills TAE, Thomas WR, Aalberse RC, Vervloet D, Chapman MD. Dust mite allergens and asthma: report of a second international workshop. *J Allergy Clin Immunol* 1992; 89: 1046-1060.
3. Johansen N, Heinig JH, Mosbech H. A new equipment for standardized dust sampling. XVII Nordic Congress in Allergology, Aarhus, May 17-19, 1990: p43.

4. Platts-Mills TAE, Hayden ML, Chapman MD, Wilkins SR. Seasonal variation in house dust mite and grass pollen allergens in dust from the houses of patients with asthma. *J Allergy Clin Immunol* 1987; 79: 781-791.
5. Luczynska CM, Li Y, Chapman MD, Platts-Mills TAE. A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, *Der pI* and *Der fI*. *J Immunol Methods* 1989; 118: 227-235.
6. Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TAE. Monoclonal anti-bodies to the major feline allergen *Fel dI*. II: Single step affinity purification of *Fel dI*, N-terminal sequence analysis, and development of a sensitive two-site immunoassay to assess *Fel dI* exposure. *J Immunol* 1988; 140: 812-818.

## Address

ALK Allergologisk Laboratorium A/S
Bøge Allé 10-12
DK-2970 Hørsholm
DENMARK

Tel: (45) 45 76 77 77
Fax: (45) 45 76 51 52

# Module Co-ordinators

### *Modules 1.1-1.4:*

These questionnaires, with the exception of the slightly changed questionnaires on demographic characteristics, have also been used for the investigation of 6-7 year olds in ISAAC Phase I.

### ***Module 2.1:*** *Additional respiratory questions*

Dr Michael Burr
Dept Of Public Health Medicine
Temple of Peace and Health
Cathays Park
Cardiff CF1 3NW
UNITED KINGDOM

### ***Modules 2.2-2.4:*** *Questionnaires on disease management*

Dr Colin Robertson
Department of Thoracic Medicine
Royal Children's Hospital
Parkville
Victoria 3052
AUSTRALIA

Tel: (61) 3 345 5844
Fax: (61) 3 349 1289
Email: cfrob@cryptic.rch.unimelb.edu.au

### ***Module 2.5:*** *Risk factor questionnaire*

PD Dr med Stephan K Weiland, MSc
Institute of Epidemiology and Social Medicine
University of Münster
Domagkstraße 3
D-48129 Münster
GERMANY

Tel: (49) 251 83 55332
Fax: (49) 251 83 55300
Email: weilans@uni-muenster.de

***Module 3.1:*** *Examination for flexural dermatitis*

Dr Hywel C Williams
Department of Dermatology
University Hospital Queens Medical Centre
Nottingham NG7 2UH
UNITED KINGDOM

Tel: (44) 115 970 9908
Fax: (44) 115 970 9003

***Modules 3.2 and 3.5:*** *Skin prick tests for atopy and Serum IgE*

PD Dr med Erika von Mutius
Kinderklinik der Universität im
Dr von Hauner'schen Kinderspital
Lindwurmstraße 4
D-80337 München 2
GERMANY

Tel: (49) 89 5160 2709
Fax: (49) 89 5160 4452
Email: erika.von.mutius@kk-i.med.uni-muenchen.de

***Module 3.3:*** *Bronchial responsiveness to hypertonic saline*

Dr Colin Robertson
Department of Thoracic Medicine
Royal Children's Hospital
Parkville
Victoria 3052
AUSTRALIA

Tel: (61) 3 345 5844
Fax: (61) 3 349 1289
Email: cfrob@cryptic.rch.unimelb.edu.au

***Module 3.4:*** *Blood sampling and frozen storage*

Prof Bengt Björkstén
Department of Paediatrics
University Hospital
S-581 85 Linköping
SWEDEN

Tel: (46) 13 22 13 31
Fax: (46) 13 14 82 65

***Module 3.6:*** *Storage of dried blood spots for genetic analyses*

Dr Innes Asher
Department of Paediatrics
Faculty of Medicine and Health Science
University of Auckland
Private Bag 92019
Auckland
NEW ZEALAND

Tel: (64) 9 373 7599 X 6451
Fax: 64) 9 373 7486

ISAAC Phase II modules are available on request from:

PD Dr med Stephan K Weiland, MSc

Institute of Epidemiology and Social Medicine
University of Münster
Domagkstraβs 3
D-48129 Münster
GERMANY

Tel: 49 (0) 251-83-55332
Fax: 49 (0) 251-83-53300
Email: weilans@uni-muenster.de