

Rapid one-step assays for on-site monitoring of mouse and rat urinary allergens

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Allergy to rodent proteins is common among laboratory animal workers. Sensitive methods to measure exposure to these allergens have been developed. These assays are, however, expensive, time-consuming, and require a laboratory facility and methodological expertise. A simple method to screen for allergen spread, or to test whether hygiene standards are maintained, would be useful. Lateral flow immunoassays (LFIA) are especially suited for field settings; the tests are simple and results are visible within minutes. LFIA were developed for detection of the rodent urinary allergens Mus m 1 and Rat n 1. Pilot studies were performed in animal facilities in three countries using both extracts from airborne dust samples and samples collected by wiping surfaces. For comparison and determination of sensitivity, the concentrations of rodent urinary allergens in the samples were also measured using enzyme immunoassays (EIAs). The LFIA for rat and mouse urinary allergens had a detection limit of 31 pg allergen per mL in a buffer system with purified allergen standards. Results of environmental dust extracts tested in LFIA correlated well with levels obtained using EIAs. Spread of rodent allergens, or non-adherence to hygiene around laboratory animal facilities, may aggravate rodent allergy. Using a simple, sensitive one-step assay, allergens can be detected to prevent allergen exposure. The results reveal that the rapid assays are suited for on-site demonstration of exposure to rodent allergens, and thus, useful in occupational hygiene practice.

Introduction

Laboratory animal allergy (LAA) is a common occupational disease among workers in animal laboratory facilities. Relationships between exposure and sensitization to rodent urinary

allergens in animal facilities and subsequent development of occupational asthma and rhinitis have been studied extensively.^{1–4}

Recent studies have questioned the methods of exposure measurement and the associations made between allergen exposure and disease development. According to Pacheco⁵ the dose–response relationship among laboratory animal exposure, sensitization and symptoms is still subject to further research to better control laboratory animal exposures. LAA or rodent allergen exposure is not restricted to laboratory workers with direct contact to rodents; indirect exposure to (laboratory) animal allergens may be as risky for sensitization as direct exposure.⁶ Krop *et al.*⁷ showed that family members of animal laboratory workers appear to be at risk for rodent allergen exposure when those allergens are transferred to their homes.

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Environmental impact

Spread of rodent allergens in people's vicinity (laboratories, schools or homes), or non-adherence to hygiene around laboratory animal facilities, can cause or may aggravate rodent allergy. This research provides a rapid and simple tool to be used by non-skilled users to monitor allergen contamination in their environment. Direct measures can be taken to remove or diminish the allergen sources and as a consequence to prevent allergen exposure.

Rodent allergen exposure is known to occur in non-occupational settings as well. Several studies have reported the presence of mouse allergens at schools and day-care centres.^{8,9} Sheehan *et al.*¹⁰ particularly studied exposure of asthmatic children and found higher levels of mouse allergens at various urban elementary schools compared to the home situations. According to Phipatanakul *et al.*¹¹ early mouse exposure was associated with early wheeze and atopy later in life. Matsui *et al.*¹² linked environmental mouse allergen exposure of mouse-sensitized inner-city preschool children with a higher rate of asthma morbidity among these children. Allergen monitoring at *e.g.* schools or animal facilities can be beneficial to diminish exposure and sensitization to airborne and settled allergens.

Lateral flow immunoassays (LFIAs) were first developed for pregnancy tests, but have since been in use for numerous applications in *e.g.* medical diagnostics, food and feed safety, and environmental control.^{13,14} Lateral flow assays, with the rapid qualitative or semi-quantitative answer to a question within minutes, are also an attractive option for hygiene purposes. These assays are especially suited for field settings and for use by non-laboratory-skilled persons, such as teachers or animal handlers. Surprisingly, the analytical sensitivity of these one-step tests can also be very high: up to 10–16 mol L⁻¹ as reported by Gordon and Michel.¹⁵ Based on the assay results, measures can be taken to decrease the presence of these allergens and effectiveness of these actions can be assessed directly. LFIAs have been applied to detect both home and occupational allergens. For use in homes, Tsay *et al.*¹⁶ developed a rapid method to detect dust mite allergens semi-quantitatively in samples collected with an integrated dust collection and extraction device. Koets *et al.*¹⁷ developed a rapid LFIA for fungal alpha-amylase monitoring at the workplace with sensitivity in the low picomolar range by using the very sensitive carbon nanoparticles as a label.¹⁵ This assay was used in a field study evaluating the performance of the testing device at the workplace, demonstrating its value in occupational hygiene surveys.¹⁸ Recently, Röder *et al.*¹⁹ evaluated commercial lateral flow devices as a screening tool for monitoring nut allergen cross-contamination in shared equipment at industrial bakeries. Used LFIAs appeared as sensitive as commercial EIAs for the specific allergens and were seen as a useful tool for sanitation in the food manufacturing industry.

Since rodent allergens cause and aggravate allergy and asthma both in homes and at work, our aim was to develop a LFIA to detect the presence of these allergens semi-quantitatively. In this study, we present two rapid one-step assays for direct on-site detection of the mouse and rat urinary allergens, Mus m 1 and Rat n 1 (see Fig. 1), respectively. The test is easy to perform requiring only two simple steps for sampling and elution and no expensive equipment. Pilot studies were performed in animal facilities in three countries using both extracts from airborne dust samples and surface wipe samples.

Materials and methods

Reagents

For development of LFIAs, antibodies against the Rat n 1 and Mus m 1 rodent urinary allergens were obtained from Mabtech

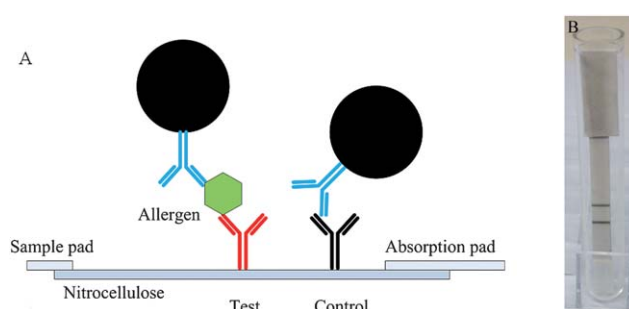


Fig. 1 LFIA test format. Panel A: a schematic view of an LFIA. Carbon-conjugated antibodies against the rodent allergens are resolved when the sample is applied to the tube. If allergen is present, a sandwich will be formed with specific antibodies at the test line and a black line will appear. As a test control anti-mouse or anti-rabbit IgG recognizing antibodies are included. Panel B: a real tube LFIA.

AB (Nacka Strand, Sweden). Control line immune reagents, goat anti-mouse IgG and goat anti-rabbit IgG, were from Jackson ImmunoResearch (Soham, UK). Chemicals used in the development of the LFIAs were analytically pure and obtained from Merck (Darmstadt, Germany). Bovine Serum Albumin (BSA) was purchased from Sigma (Zwijndrecht, The Netherlands).

Rodent allergen standards

The rat and mouse urinary allergen standards, Rat n 1 and Mus m 1, respectively, were prepared from dialysed metabolic cage urine from post-pubertal male Sprague-Dawley rats and NMRI mice as described previously.^{20,21}

LFIA development

Affinity purified batches of the monoclonal antibodies against Rat n 1, Mab-1 and Mab-6, and the polyclonal Mus m 1 antibodies were buffer exchanged with borate buffer 5 mM, pH 8.8 using centrifugal filter devices (50 000 NMWL, Millipore, Billerica, USA). Protein yields of antibody fractions were estimated by the bicinchoninic acid (BCA) assay kit as described by the manufacturer (Pierce, Rockford, USA).

Antibodies, Mab-1 for the RUA assay and polyclonal anti-Mus m1 for the MUA assay, were conjugated to colloidal carbon nanoparticles as described by Koets *et al.*¹⁷ Carbon-antibody detection conjugates were resuspended in buffer as a 0.25% suspension (w/v) and stored in the dark at 4 °C.

The capture antibodies, Mab-6 for RUA and polyclonal anti-Mus m1 for MUA assay, were applied to nitrocellulose membranes in a line format using a Linomat IV (CAMAG, Muttens, Switzerland). Capture antibodies were sprayed at a concentration of 400 ng per 5 mm. As a positive control, a line of goat anti-mouse IgG or goat anti-rabbit IgG for the MUA or RUA assay, respectively, was applied at a concentration of 100 ng per 5 mm. The lines were immobilised at a distance of 13 mm for the specific line and 18 mm for the control line to the beginning of the strip. The membranes were dried for at least 2 h at 37 °C. The membranes were cut into individual strips of 5 mm width by the Cutter 4000 (BioDot, Chichester, UK). Fig. 1 shows both a schematic view of the LFIA and a real tube LFIA. The assay components were assembled as described previously.¹⁷

Rapid immunoassay

Samples (total volume 100 μL) were applied in running buffer (0.1 M borate buffer, pH 8.8; 1% (w/v) BSA; 0.1% (w/v) Tween 20 and 0.02% (w/v) NaN_3), directly on top of the dried carbon conjugate at the bottom of the tube. Subsequently, the membrane with attached absorbent pad was placed in the tube to start the assay. Upon running a sample in this simple tube format the result should be read within 30 minutes maximally, although a black line may be visible within some minutes if the sample contains the allergen above certain concentrations. Test lines were scanned with a flatbed scanner (Epson Perfection 3200 Photo) and the line intensities (pixel grey volume) were analysed with TotalLab (Nonlinear Dynamics). Curve fitting was done with Sigmaplot 10 (Systat Software).

Pilot studies at laboratory animal facilities

Assay specificity and ease of use were assessed by a pilot laboratory trial in animal house facilities in Sweden, Germany and The Netherlands. Comparisons were made between levels of rat and mouse allergens, obtained by using EIA and LFIA. Both airborne dust samples captured on filters and wipe samples obtained by wiping surfaces with filters were tested. The EIAs used had been developed by the laboratory performing the assays.^{20–22} The Institute of Prevention and Occupational Medicine of The German Social Accident Insurance (IPA, Bochum, Germany) performed the EIAs using the standards and polyclonal antibodies from Institute for Risk Assessment Sciences (IRAS, Utrecht, The Netherlands) with the amplification protocol from Karolinska Institutet (KI, Sweden, Stockholm).²³ LFIA results were semi-quantified on a 0 to 5 (no line–very intense) scale after a maximum of 30 minute assay incubation.

Settled dust wipe samples

Filter paper (Whatman, Middlesex, UK, no. 4 with 5.5 cm diameter) was used to wipe an area as large as a regular-sized paper (A4-size, simple comparison size) at several areas in the lab animal facility, *e.g.* animal housing, work desk and canteen table. Dust samples were collected with a filter that had been pre-wetted with 0.5 mL Phosphate Buffered Saline (PBS) containing 0.05% (v/v) Tween 20. Filters were placed in a 15 mL tube and eluted with 6 mL running buffer, by shaking it by hand a couple of times followed by vortexing for 10 seconds at maximum speed. At IPA extractions were performed for approximately 5 minutes at room temperature. Supernatants were applied directly to the assay. To investigate the most optimal extraction conditions KI tested some variations within the extraction protocol; also dry filters were used. Elution was done with 10 mL running buffer and extracts were incubated by end-over-end rotation for 10 minutes. Some samples were used directly, *i.e.*, without centrifuging; others were centrifuged at $1000\times g$ for 10 minutes.

Airborne dust samples

At KI air samples were collected during work with animals on 1 μm pore size polytetrafluoroethylene filters (PTFE, Millipore, Sweden) in Institute of Occupational Medicine (IOM, Edinburgh, UK) inhalable dust sampling heads applying an airflow of

2 L min^{-1} (SKC Inc, USA) fastened to the breathing zone using person-carried pumps. Samples were collected during different sampling times varying from 30 minutes to 5 hours. The filters were incubated in 1 mL PBS, 1% (w/v) BSA, 0.5% (v/v) Tween 20, and 0.15% (v/v) Kathon and rotated for 2 h at room temperature. After elution of the allergens, BSA was added to 1% (w/v final concentration) and eluates were stored at -20°C until analysis. IRAS collected ambient airborne dust samples in lab animal facilities with the parallel sampling unit (PSU) used previously in the bakery environment,²⁴ applying PAS6 inhalable dust sampling heads with an airflow of 2 L min^{-1} . Samples were collected during different sampling times varying from 30 minutes to 8 hours. Filters used in this study were extracted with PBS containing 0.05% Tween 20 and incubated for 1 hour using an end-over-end roller. Extracts were stored at -20°C until analysis. For LFIA, filter extracts were mixed with an equal volume of double-strength running buffer and 100 μL of this diluted sample was added to the assay.

Results

Dose–response curves

The sensitivity of the LFIAs for mouse and rat urinary allergen detection was determined by applying serial dilutions of the allergens to the tube-LFIAs. Purified rodent allergens Mus m 1 and Rat n 1 were used as a standard. The resulting assay test lines were scanned and the pixel grey volumes corresponding to the line intensities were rendered. Test lines at the lowest allergen concentrations could be observed by the eye. The results of three individual assays were used for every data point; the means and standard deviations are depicted in the dose–response curves (see Fig. 2A and B). The Limit of Detection (LOD) of the assays was the pixel grey volume of the blank assay (no allergen standard present) plus three times the standard deviation. This resulted in an LFIA with a detection limit of 31 pg Mus m 1 per mL for MUA-LFIA and 31 pg Rat n 1 per mL for RUA-LFIA.

Rat and mouse allergen detection by LFIA

To assess the practical use of the developed LFIAs, a three-laboratory trial was performed on both airborne and settled dust samples. Wipe samples of settled dust were collected from various places in laboratory animal facilities. Samples were taken at places with expected high (mouse and rat rooms) and low allergen levels (offices). Since both rats and mice were housed at these animal facilities, dust extracts were applied to both RUA and MUA LFIAs. Dust extracts tested in LFIA varied in allergen concentration from 1 to 6410 ng MUA per mL extract, as determined by EIA. Samples with MUA concentrations higher than 1500 ng MUA per mL were assigned a lower line intensity in LFIA by the assay observers (*i.e.*, a lower score) than samples with a high concentration but lower than 1500 ng MUA per mL. Nevertheless, these $>1500\text{ ng mL}^{-1}$ samples were still scored positive. For RUA, allergen concentrations ranged from 0.2 to 1167 ng RUA per mL extract, and no visible decrease in signal has been observed by the readers as an effect of high levels of RUA within the dust extracts. Distinct lines appeared in both assays, indicating the presence of both allergens in the various

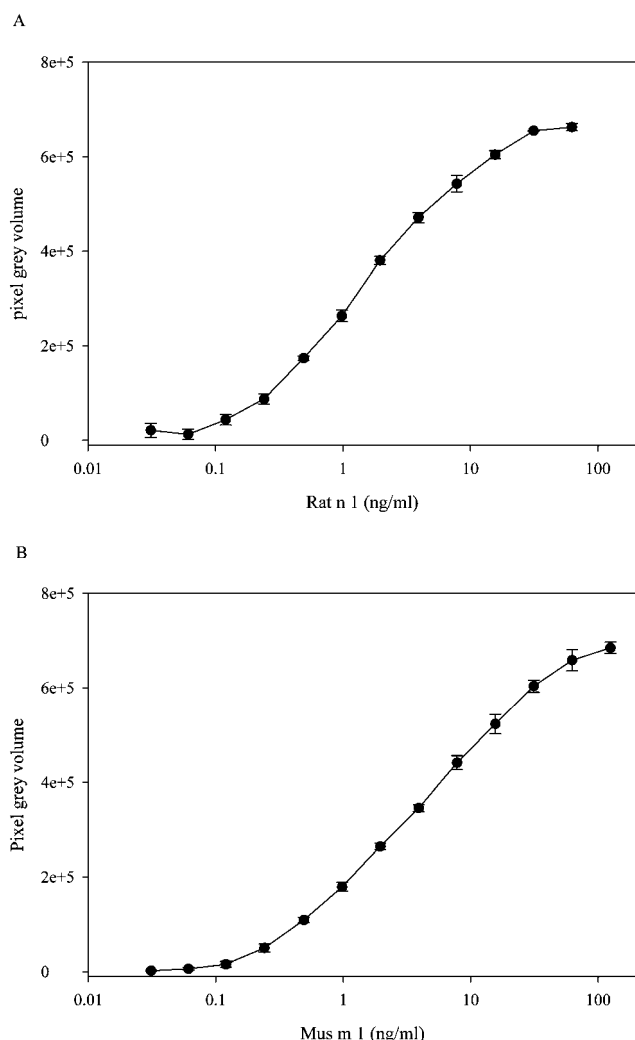


Fig. 2 Dose-response curves of the RUA tube-LFIA (panel A) and the MUA tube-LFIA (panel B). Dilution series (from 62.5 to 0.03 ng mL⁻¹) of the standard Rat n 1 in buffer and a dilution series (from 125 to 0.03 ng mL⁻¹) of the standard Mus m 1 in buffer. The curves show the calculated pixel grey volumes of an LFIA after flatbed scanning and image analysis. Each data point represents the mean and standard deviation of three individual assays.

rooms. A selection of these assay strips is shown in Fig. 3. These results were confirmed by EIA values (see Table 1).

Allergen levels in airborne dust samples are in general low compared to settled dust samples. Although the LFIA were developed initially for allergen detection in settled dust, the



Fig. 3 LFIA results of wiped filter extracts. Samples were taken in an animal facility in Germany (Table 1). After extraction of the samples, eluates were applied to both a RUA (left membrane) and a MUA (right membrane) LFIA. Table 1 shows a comparison between LFIA results and allergen levels of the extracts obtained with EIA. The upper line (C) of each test: control line, the lower line of each test (T): test line.

Table 1 EIA results of wiped samples taken in a laboratory animal facility. RUA and MUA levels in filter eluates from wiped samples taken at various places in a laboratory animal house were determined using EIA (IPA, Bochum, Germany). Fig. 3 shows the LFIA results of these filter extracts

Sample	Room	Surface	RUA ^a / ng mL ⁻¹	MUA ^b / ng mL ⁻¹
1	Mouse and rat room	Work table	1.167	1584
2	Mouse room	Work table	18.7	2750 ^c
3	Corridor to different mouse rooms	Small work board	35.2	308.8
4	Rat room	Trolley	6.8	7.9
5	Rat room	Shelf in rat rack	170.9	137.1
6	Cage wash room	Trolley	50.9	24.0

^a RUA EIA according to IPA (Bochum, Germany). ^b MUA EIA according to IPA (Bochum, Germany). ^c Dust sample positive in LFIA, but line intensity decreased due to the high-dose-hook effect.

suitability of this assay format for use with airborne dust extracts was tested as well by applying some of the eluates to LFIA tubes (see Fig. 4). Filter eluates varied in concentration from 0.038 to 34 ng MUA per mL extract and for RUA between 0.29 and 23.7 ng RUA per mL extract, as determined by the respective EIAs. It appeared that in some samples very low levels of allergens could still be detected in LFIA for both RUA and MUA. RUA extracts containing 0.52 ng RUA per mL still gave a distinct line and 0.15 ng MUA per mL was positive in the MUA LFIA.

Evaluation LFIA results

In total 66 samples of various origins were tested with the mouse allergen tube-LFIA. LFIA results were semi-quantified on a 0 to 5 scale after a maximum of 30 minute assay time (see Fig. 5A). In EIA 63 samples showed detectable MUA allergen levels and 60 of them were positive in LFIA as well (95%). Three samples that tested negative in EIA were also negative in LFIA.

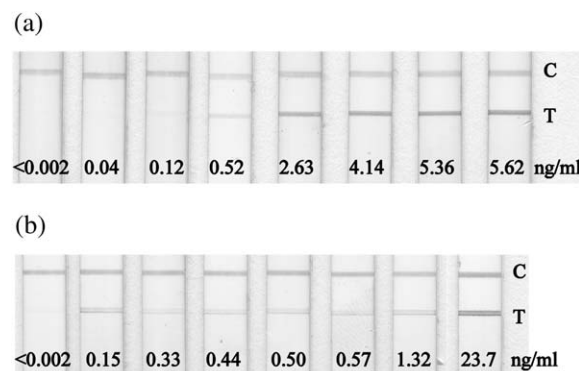


Fig. 4 LFIA results of air samples taken in an animal house facility (Sweden). Samples were collected using person-carried pumps, with the air filter fastened in the breathing zone. After extraction of the samples from the filters, eluates with a range in allergen concentrations were applied to a MUA (upper panel) or a RUA (lower panel) LFIA. C: control line and T: test line.

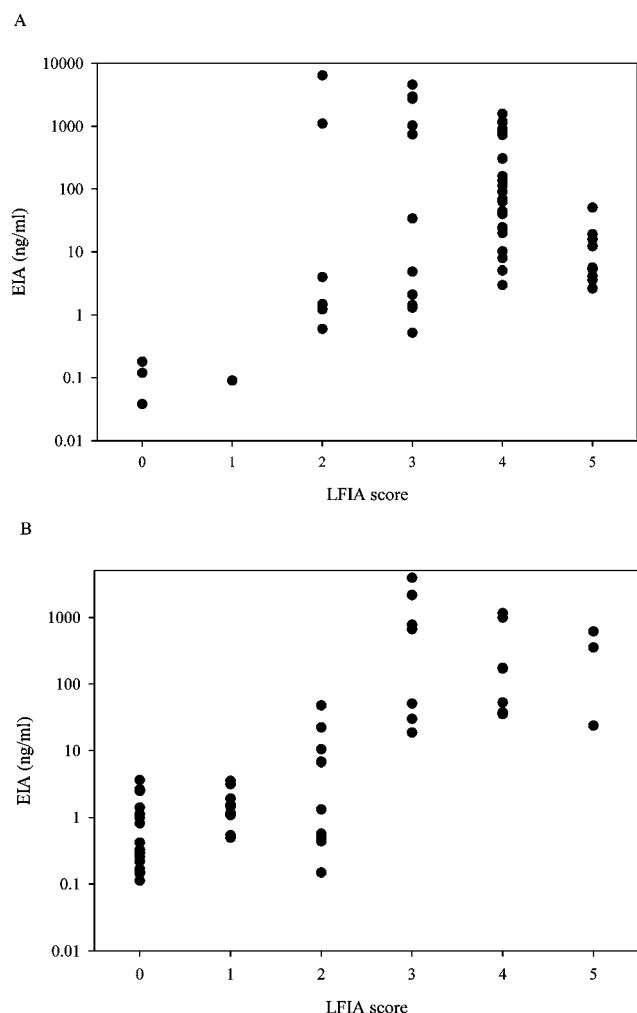


Fig. 5 Correlation between EIA and LFIA results. The LFIA line intensity is the score of observers' readings and is indicated as no line (0), weak (1) to very strong (5). Samples were taken at three animal facilities and included both wipe and air samples (panel A: MUA LFIA; $n = 66$, panel B: RUA LFIA; $n = 58$).

Using the rat allergen tube-LFIA, 58 samples were tested (see Fig. 5B). Of these samples, 55 extracts were found positive in EIA, of which 37 were positive in LFIA as well (67%). Three samples that tested negative in EIA (or below the LOD of the EIA) were also negative in LFIA. Some differences were seen comparing the RUA LFIA scores at the lower allergen concentration range, *i.e.* <5 ng RUA per mL. Below this allergen level extracts with comparable allergen concentrations scored either positive or negative in LFIA. In total 33 extracts, both wipe and air samples, had EIA rat allergen levels between 0.1 and 5 ng RUA per mL extract. Of these assays, 18 (55%) EIA-positive samples tested negative in LFIA and 10 (30%) were assigned a line intensity of 1. However, 5 filter eluates (airborne samples), having RUA concentrations between 0.129 and 1.325 ng RUA per mL eluate, were assigned a line intensity of 2 (15%) (see Fig. 4).

Discussion

We developed a rapid semi-quantitative one-step immunoassay to monitor exposure to mouse and rat urinary allergens on-site.

The LOD of the LFIA showed to be 31 pg allergen per mL in a buffer system with known standards for both MUA and RUA LFIA. Above 125 ng Mus m 1 per mL for the MUA assay and 62.5 ng Rat n 1 per mL for the RUA assay the assay signal, *i.e.*, the carbon line intensity, decreased as a result of the high-antigen-dose-hook effect. Due to the high allergen concentrations both capture and detection antibodies become saturated with allergen resulting in a reduced chance of sandwich formation between the assay components and, hence, in a decrease of line intensity.

Pilot field studies with developed LFIA have been performed at three different locations (countries). To be able to perform the tests on-site, a rapid uniform sampling method needs to be included in the assay. Therefore, dust sampling and extraction methods were investigated during the field studies. Dust samples were extracted using short extraction protocols (a maximum of 10 minutes), except for the airborne dust samples which were extracted from the filters during a 1 to 2 hour incubation. The obtained LFIA results show that by using these developed extraction methods, it was possible to detect mouse and rat allergens by LFIA in a concentration range from 0.5 to 6410 ng MUA per mL and 0.2 to 1167 ng RUA per mL extract. Based on the samples and the sampling and extraction protocols used it cannot be concluded that a particular protocol would be best. Sampling and extraction need to be further investigated regarding *e.g.* incubation times, extraction buffers and filter use, with larger numbers of samples to allow a secure statistical evaluation.

At first glance the MUA-LFIA is more sensitive than the RUA-LFIA. For the MUA assay, only 3 ($n = 66$) EIA-positive samples scored negative in a MUA LFIA. These samples contained a MUA concentration <0.2 ng MUA per mL of extract. As for the RUA LFIA at the lower RUA allergen concentrations, below 4 ng allergen per mL extract, only 15 out of 33 EIA-positive extracts were identified positive in LFIA (45%). Above this allergen concentration all RUA LFIA ($n = 22$) were positive (100%). This indicates that the 4 ng RUA per mL level would be a more realistic LOD for real field samples.

In general the level of RUA allergens in the samples appears to be lower than MUA. Only 6 MUA extracts contained less than 1 ng allergen per mL in contrast to the RUA samples where 18 samples appeared to be <1 ng RUA per mL extract. This can be accounted for by the excess of mice compared to rats housed in the animal facilities under study, *i.e.* in the German animal facility 12 000 mice and only 160 rats were housed at the time of study.

Although the line intensity of the LFIA decreased at higher allergen concentrations, a clear line was still visible in extracts containing up to 1167 ng RUA per mL and 2750 ng MUA per mL (Fig. 3). Even at 6410 ng MUA per mL a distinct line was observed (not shown). This indicates that both assays can be used for samples with rodent allergens that differ well over three orders of magnitude. For use in a field setting this is a great advantage: in most cases it will not be necessary to dilute samples to exclude a possible false-negative result due to the hook effect. However, testing of additional dilutions (1 : 10 or 1 : 100) would be useful for better estimation of the real allergen content.

Exposure to mouse and rat urinary allergens is an important risk factor for the development of (occupational) allergy and

asthma. Dose–response relationships between animal allergen levels and symptoms or sensitization do not appear to be linear. In cross-sectional prevalence studies, workers in the highest exposure group have a lower frequency of symptoms or sensitization compared with moderately exposed workers.⁵ Pongracic *et al.*²⁵ monitored the effects of rodent environmental intervention on asthma among inner-city children. Results revealed that home rodent-specific intervention led to reduced mouse allergen levels and resulted in reduced asthma-related sleep and activity disturbance. The significance of allergen reduction or elimination in exposure studies still needs to be further evaluated. Publications lack quantitative exposure information and/or results between studies cannot be compared because of different exposure (assay) parameters.²⁶ In general, to prevent and manage asthma it is recommended to reduce exposure to allergens.²⁷ As expected results from the pilot study demonstrated that rodent allergen levels were high at places where the animals were accommodated. However, rodent allergens could also be detected in wipe samples in corridors, cage washing rooms and even in offices (see also Fig. 3). To our knowledge, this is the first rapid and simple test for environmental (pre-)screening of mouse and rat allergens. Up to now, epidemiological studies have been performed using EIAs carried out at laboratories by skilled personnel.

Conclusions

In this paper we demonstrate that the developed RUA and MUA LFIA are suitable rapid methods to identify environmental allergen sources. Especially in settings which lack the technological skills for laboratory testing these assays can be introduced into occupational hygiene practice. Rodent urinary allergens could be detected in extracts of wipe and air dust samples at a low ng per mL level. Application of extracts of settled dust to such LFIA may allow direct on-site demonstration of work-related hazards of bio-allergen exposure and test the effectiveness of measures to prevent allergen exposure.

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