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A rapid test for detection of mite allergens in homes

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Summary

Background International guidelines recommend allergen avoidance for asthma management, but do not include making assessments of allergen exposure. Mite allergen exposure cannot be assumed, especially in geographical regions where climatic conditions vary.

Objective To develop a rapid test that would enable consumers to detect mite allergen in the home.

Methods A lateral flow test using gold labelled antibody for mite group 2 allergen was developed as part of a detection kit incorporating the MITEST dust sampling device. Dust samples were assayed by ELISA for group 1 and group 2 allergens and by using the rapid test. The tests were compared as indices of mite allergen exposure.

Results There was a good correlation between group 1 and group 2 levels by ELISA ($n = 349$, $r = 0.60$, $P < 0.001$). In a multi-centre study of 65 homes (263 dust samples) in five countries, there was a strong correlation between ELISA and the rapid test. Most samples with high scores in the test (43/48, 90%) contained $> 1\ \mu g/m^2$ group 2 allergen, whereas most low samples contained $< 1\ \mu g/m^2$ (50/64, 78%). Differences between mean group 2 levels of samples that scored low ($0.28\ \mu g/m^2$), medium ($1.68\ \mu g/m^2$) or high ($3.18\ \mu g/m^2$) on the test were highly significant (P 0.007 to < 0.001).

Conclusions A simple rapid test has been developed that detects mite allergen in the home within 10 min. The mite screening test should educate consumers about allergen exposure and encourage compliance with allergen-avoidance procedures. This technology has applications for the detection of other common environmental allergens.

Keywords allergen detection, asthma, environment, *in vitro* diagnostics, mite allergens

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Introduction

There is a strong association between sensitization to indoor allergens and the development of asthma [1–6]. National and international guidelines for asthma management recommend allergen avoidance as the first step in the treatment of asthma and perennial rhinitis [7–10]. These guidelines also recommended the use of patients' histories, questionnaire and IgE-mediated sensitization to mites as evidence of allergen exposure, but did not include any environmental assessment. There are obvious flaws with this approach. Patients are unaware of the degree of mite infestation in their homes and evidence of sensitization may not be indicative of current allergen exposure. Mite species, population densities and allergen levels vary depending on geographical location, temperature, humidity and housing characteristics [reviewed in 1]. Studies in Europe and elsewhere showed that mite allergen levels were lower at high altitude [11–13]. In the USA, inner city homes located in cities on the east coast (e.g. Baltimore, Cleveland, New York) had low levels of mite allergens, whereas high levels were reported in urban areas of the south (e.g. Atlanta) [3, 4, 14–16]. A study in Boston showed that mean Der f 1 levels in houses were 10- to 100-fold higher than those found in apartments in the same city [17]. The results of these epidemiological studies suggest that it is unwise to make assumptions about mite allergen levels and emphasize the need for allergen measurements to confirm exposure.

The gold standards for measuring exposure to mite allergens have been two site monoclonal antibody (mAb)-based enzyme immunoassays (ELISA) for mite group 1 or group 2 allergens [1]. However, ELISA remains a research tool and the technology is not applicable to home-based testing. The need to further educate patients and demonstrate the importance of indoor allergens in asthma has spurred the development of simple qualitative or semiquantitative tests that use allergen-specific antibodies bound to membranes in dipstick or cassette formats [18]. The DUSTSCREEN detects mite, cat and cockroach allergens on a single test strip and was designed for use in pharmacies, allergy clinics or doctors' offices, as an alternative to ELISA, but is not suitable for home use [19, 20]. The ACLOTEST dipstick uses polyclonal antibodies to mite antigens and takes 30 to 60 min to develop [21].

Here, we describe the development of a simple, rapid test for dust mite (*Dermatophagoides* spp.) group 2 allergens that uses lateral flow technology and gold labelled mAb for allergen detection. The test incorporates a device that can be used to collect and extract dust from a defined area and the results correlate with ELISA. This technology will enable healthcare

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professionals and patients to check dust mite levels in the home and should improve patient education and compliance with allergen-avoidance procedures.

Materials and methods

Lateral flow test for mite group 2 allergens

The principle of the test is outlined in Fig. 1. Capture anti-Der p 2 mAb (clone 1D8) at 1 mg/mL in 0.01% BSA was striped onto nitrocellulose membrane (0.5 μg/cm) as a line positioned 0.9 cm from the base of the membrane. A second anti-Der p 2 mAb (clone 7A1) labelled with 40 nm colloidal gold particles was incorporated into a sac located at the end of the nitrocellulose strip and overlaid with a paper wick. Allergen sample or dust extract (vol. *c*. 150 μL) prepared in 1% BSA-PBS-T (phosphate buffered saline, pH 7.4 containing 0.05% Tween 20) was applied to the wick and released the gold labelled mAb, which immediately bound to any mite group 2 allergen in the sample. The allergen/mAb complex diffused along the nitrocellulose until it reached the line of capture mAb 1D8, where it was deposited and visualized as a solid red line. The line developed within 10 min and the intensity of the colour was proportional to the allergen concentration in the sample. The test strip was also coated with three concentrations of a protein-gold conjugate (OD2-30), which served as 'indicator' lines, against which the allergen line was compared to obtain low, medium and high estimates of group 2 allergen.

Mite group 1 and group 2 allergen levels in dust samples

The correlation between group 1 and group 2 allergens was assessed by comparing allergen levels in 349 dust samples from nine locations in different parts of the world: Charlottesville and Baltimore, USA; Manchester, UK; Ribeirao Preto, Brazil; Wellington, New Zealand; Sydney, Australia; Amsterdam, the Netherlands; Strasbourg, France; and Stockholm, Sweden. These dust samples had been collected from carpets or bedding and prepared by extracting 100 mg fine dust in 2 mL PBS-T [1]. Samples were assayed by ELISA for Der p 1 and Der f 1, as described previously [22]. Mite group 2 allergen was measured using a single, cross-reactive ELISA that measures both Der p 2 and Der f 2 [23]. Group 2 assays were quantified using a Der p 2 standard containing 5000 ng/mL Der p 2 (Indoor Biotechnologies Inc., Charlottesville, VA, USA).

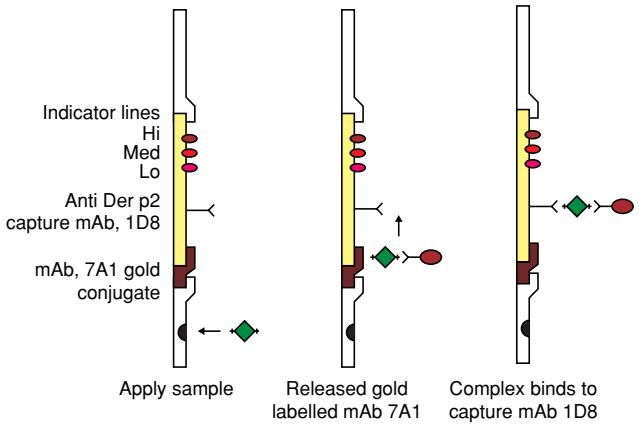

Fig. 1. Lateral flow test for mite group 2 allergens.

Multi-centre study evaluating use of the MITEST dust sampler and the lateral flow test: correlation with ELISA

The MITEST dust sampler is an integrated dust collection and extraction device that enables dust to be sampled on a unit area basis and extracted without the need for dust handling (MITEST Ltd, Dublin, Eire). The device consists of a moulded plastic housing containing a disposable, inner nylon filter that fits on to the suction end of a vacuum cleaner wand [24]. The nylon filter is semipermeable and permits air flow whilst allowing dust to be collected. An area of 0.25 m^2 was vacuumed for 2 min, after which the lower end of the device was capped and 10 mL PBS-T added to the sampler. The top of the collector was capped, shaken for 1 min, and allowed to stand for 4 min. The dust extract was then removed for analysis by rapid test or ELISA.

A multi-centre study was designed to assess the validity of allergen measurements on dust samples that were collected by MITEST and analysed using the rapid test. Each centre collected up to four dust samples (bedding, bedroom floor, living room carpet and soft furnishings) from 10 to 18 homes using the MITEST collector and analysed the dust extracts by rapid test and ELISA. The rapid tests were scored low, medium or high based on the indicator lines on the test. The five participating centres were located in: Charlottesville and Baltimore, USA; Sydney, Australia; Strasbourg, France; and Stockholm, Sweden. Allergen levels were determined by ELISA (expressed as μg/m^2) and compared with the line intensity of the rapid test. Geometic mean (GM) values and 95% confidence intervals for mite group 2 allergen levels were calculated for each intensity score. Statistical comparisons between the means were made using the Mann–Whitney test.

Results

Correlation between mite group 1 and group 2 allergen levels

Most assessments of mite allergen exposure have been carried out using species-specific ELISA tests for either Der p 1 or Der f 1. The rationale for developing the lateral flow test for group 2 allergen was to enable allergens produced by both *D. pteronyssinus* and *D. farinae* to be detected in a single test. Unlike Der p 1 and Der f 1, most mAb to group 2 allergens are cross-reactive and can be used to detect both Der p 2 and Der f 2 [23]. The relationship between group 1 and group 2 allergen levels was compared by analysing 349 dust samples collected in nine locations from different parts of the world (the USA, Europe, Brazil, Australia and New Zealand). Linear regression analysis showed a significant correlation between ELISA for group 1 (Der p 1 + Der f 1) and group 2 allergens ($n = 185$, $r = 0.48$, $P < 0.001$, Fig. 2A). Der f 1 was not assayed at four locations where *D. pteronyssinus* was the dominant mite species. The correlation between Der p 1 and Der p 2 levels at these locations was also significant ($n = 164$, $r = 0.68$, $P < 0.001$, Fig. 2b). The strength of the correlation varied somewhat between countries. There was a strong correlation between Der p 1 and Der p 2 in dust samples from Wellington, New Zealand, and from

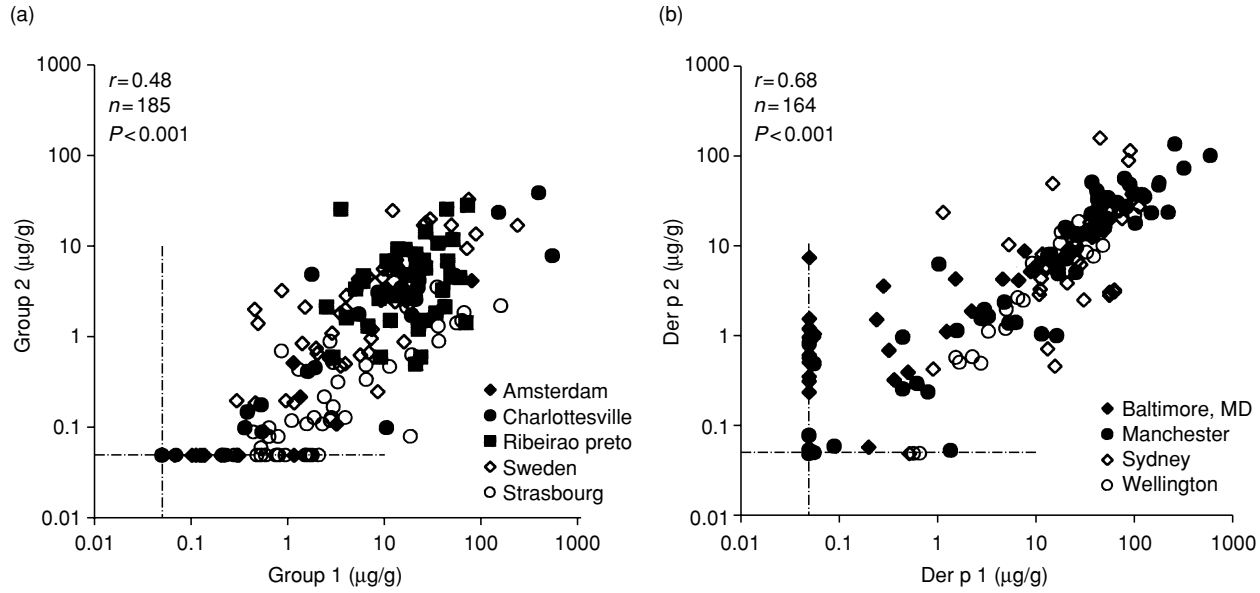

Fig. 2. Correlation between group 1 and group 2 allergen levels. Dust samples from Amsterdam, the Netherlands, Charlottesville, VA, USA, Ribeirao Preto, Brazil, Stockholm, Sweden, and Strasbourg, France, were analysed for group 1 (Der p 1 and Der f 1) and group 2 by ELISA and the results were compared by linear regression analysis (a). Samples from Baltimore, MD, USA, Manchester, UK, Sydney, Australia, and Wellington, New Zealand, were analysed for Der p 1 and Der p 2 (b). The lower level of sensitivity of each assay is indicated (–·–).

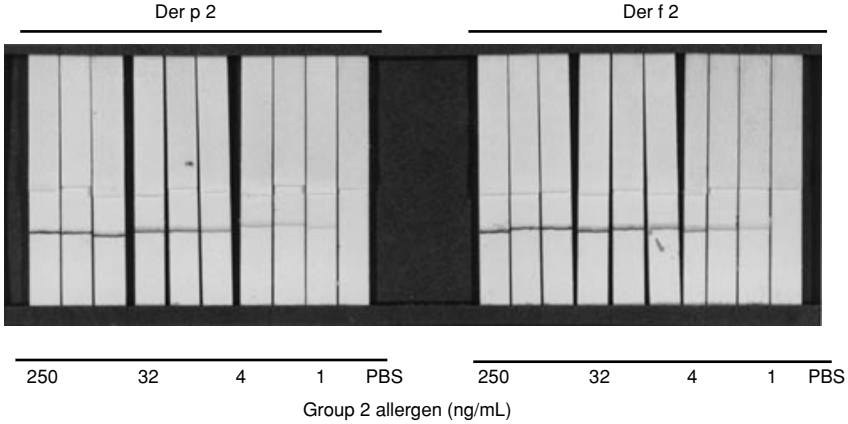

Fig. 3. Comparison of allergen standards in the lateral flow test for group 2 allergens. Serial dilutions of Der p 2 and Der f 2 standards (1 to 250 ng/mL) were analysed using rapid test strips (without indicator lines).

Manchester, UK, with correlation coefficients of 0.93 and 0.83, respectively. Least significant correlations were obtained for samples from Ribeirao Preto, Brazil, and Strasbourg, France, ($r = 0.29$ and 0.40, respectively). Although the overall correlation between group 1 and group 2 levels was statistically significant ($n = 349$, $r = 0.60$, $P < 0.001$), there was a broad spread of data and the group 1 : group 2 ratio varied from approximately twofold to more than tenfold in some samples (Fig. 2a, b).

Lateral flow test for mite group 2 allergen

Comparison of the reactivity of *D. pteronyssinus* or *D. farinae* allergen standards used for ELISA in the lateral flow test showed that both Der p 2 and Der f 2 were detected with similar levels of sensitivity (*c.* 1–2 ng/mL, Fig. 3). To evaluate the test performance in the home, a multicentre study was carried out in five locations: Charlottesville, Baltimore, Stockholm, Strasbourg and Sydney. Each centre collected three to four dust samples (bedding, bedroom floor, living room carpet and soft furnishings) from 10 to 18 homes. The samples were collected using the MITEST dust collection and extraction device and analysed by rapid test. The recommended procedure for sampling using the MITEST device was to sample four separate areas, approximately the size of one sheet of letter size paper, for 30 s each (total *c.* 0.25 m^2, sampling time 2 min) prior to extraction. The weight of dust collected in selected bed (mattress), sofa and carpet samples from central Virginia using the MITEST collector was as follows: bed, $n = 12, 140 \pm 85$ mg; sofa, $n = 7, 142 \pm 151$ mg; carpet, $n = 27, 660 \pm 446$ mg.

Each centre graded the rapid test line intensity as negative or as low, medium or high relative to the coloured indicator lines on the test (Fig. 4) and the results were compared with ELISA values (expressed as $\mu g/m^2$) on the same samples (Fig. 5). Dust samples from different centres showed similar patterns of reactivity in the rapid test, with a progressive increase in allergen levels from low to high test scores. Most of the samples that gave a high score in the rapid test contained $> 1\ \mu g/m^2$ group 2 allergen (43/48, 90%), whereas most of the low scoring samples

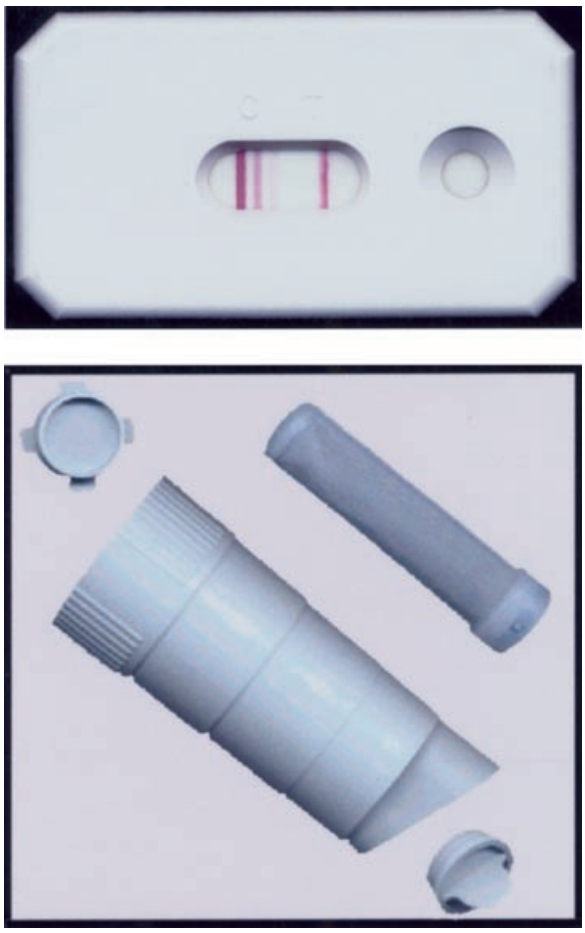

Fig. 4. Mite allergen detection kit. Upper panel: lateral flow test with a dust sample showing a strongly positive test (red line at the T position) and the high, medium and low indicator lines. Lower panel: the MITEST dust sampling and extraction device.

contained $< 1\,\mu g/m^2$ (50/64, 78%). The test showed a low rate of false negative reactions: only 3/95 samples (3.15%) that were negative on the rapid test showed $> 0.1\,\mu g/m^2$ group 2 by ELISA. The GM values for group 2 allergen between scored samples were as follows: low, $0.28\,\mu g/m^2$, confidence interval (CI) 0.186 to 0.432; medium, $1.68\,\mu g/m^2$, CI 1.210 to 2.337; high, $3.18\,\mu g/m^2$, CI 2.309 to 4.367 (Fig. 5). Non-parametric (Mann–Whitney) statistical comparisons showed highly significant differences between the GM group 2 levels for each rapid test score: negative to low, $P < 0.001$; low to medium, $P < 0.001$; medium to high, $P = 0.007$.

Discussion

We have developed a simple lateral flow test kit for detecting mite allergen in the home which measures the group 2 *Dermatophagoides* spp. allergens. The rationale for measuring group 2 was that the test would not be influenced by the mite fauna in a particular location. Most epidemiological studies have used group 1 measurements as indices of allergen exposure [1]. Therefore, it was important to investigate the relationship between group 1 and group 2 levels in dust samples from different world-wide locations. Overall, there was a good correlation, though the ratio of group 1 to group 2 varied by up to tenfold. Previous studies from Japan and from Brazil reported mean group 1 : group 2 ratios of 0.8 and 1.0, respectively [25, 26]. The mean ratios in our studies ranged from 1.3 (Sweden) to 4.6 (Brazil). The factors that affect the production of the group 1 and group 2 allergens by mite populations are poorly understood. Mites produce *c*. 20 faeces/day containing group 1 allergen [27]. Sequence homology and three dimensional structural data suggest that the group 2 allergens may be related to moulting proteins or have a reproductive function [28–30]. Thus while faeces production may be continuous, we speculate that group 2 allergens may be produced at higher levels in rapidly growing mite populations or during certain stages of mite growth, which would affect both the extent to which they accumulate in dust and the group 1 : group 2 ratio. In the USA the ratio may also be affected by differential growth of *D. pteronyssinus and D. fairnae*, which varies with different geographical and climatic locations [31].

Lateral flow technology is used extensively in human *in vitro* diagnostics, but has not been used for allergen detection. Assessment of allergen exposure presents special problems because, unlike pregnancy or HIV tests, allergen tests require methods for collecting and extracting dust prior to analysing the sample. We have overcome these problems by using a simple, disposable plastic collector (MITEST) that enables dust samples to be collected and extracted in a uniform manner within 5 min. This collector will be of general use for indoor allergen sampling. It has an inner filter of 40 μm that allows airflow while retaining allergens and can be used to sample either on a weight or unit area basis. A position paper of the British Society for Allergy and Clinical Immunology recommended recoverable allergen per unit area as a better index of allergen exposure (than allergen per gram dust) and an excellent correlation between recoverable allergen per unit area and per weight was previously reported for mattress dust samples [7, 32].

Most consumer-based lateral flow tests are designed to show either a positive or a negative result. Allergen exposure may vary by orders of magnitude, hence the mite allergen test was designed with indicator lines to provide the consumer with estimates of exposure. The indicator lines are intended as a guide to help patients check mite allergen exposure; they are not an alternative to ELISA and should not be interpreted to provide absolute exposure measurements. The advantage of the indicator lines is that they are pre-printed on the test and allow the consumer to make a direct comparison of the colour intensity of their dust sample with the indicator lines. Recently, a 'wipe' test for mite allergen was reported that also uses lateral flow technology [33]. This test does not include indicator lines, but has a grading scale in which classes 2 and 3 appear to correspond to the medium and high intensity scores on the rapid test.

The advantages of the lateral flow test kit are that it includes a convenient sampling and extraction device, which samples a defined unit area, and the test is designed for home use: sampling, extraction and test results can be completed within 10 to 15 min. Patients using the test could obtain useful information about mite allergen exposure by sampling two to four sites within a home. Although further studies are needed to establish risk levels of exposure for the group 2 allergens, our results suggest that mite-allergic patients whose test results are high

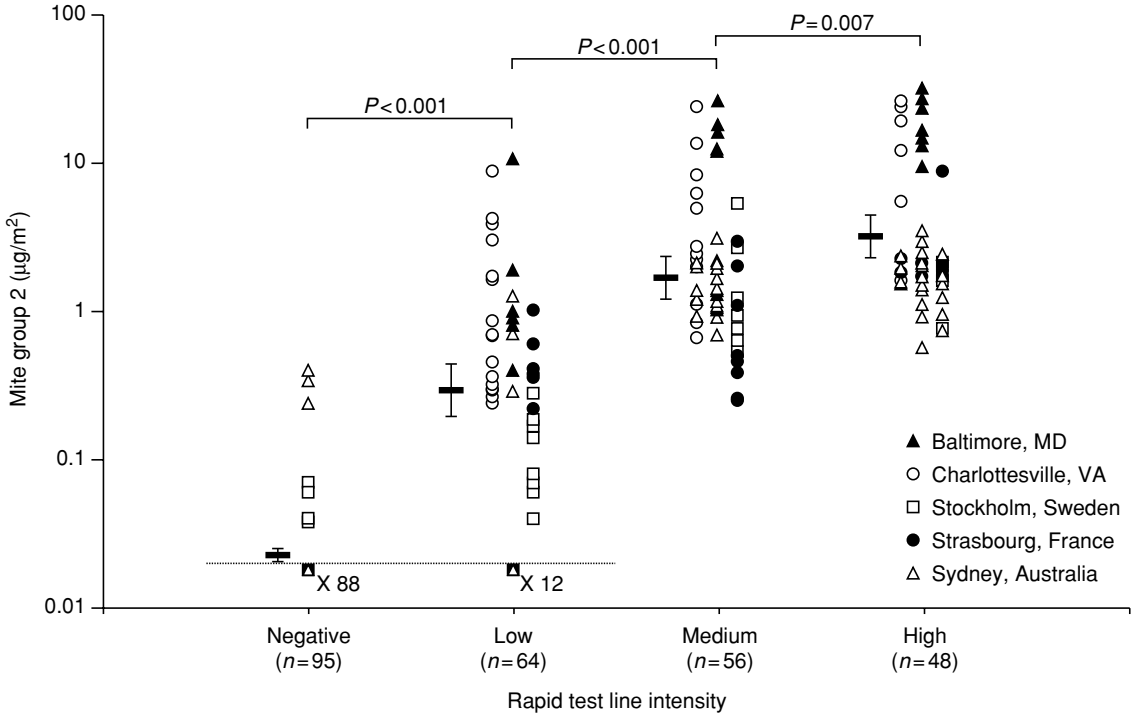

Fig. 5. Comparison of lateral flow test results and ELISA. The figure shows rapid test line intensity and ELISA values of dust samples ($n = 263$) collected with the MITEST sampler in: Charlottesville, VA, USA ($n = 73$); Baltimore, MD, USA ($n = 48$); Stockholm, Sweden ($n = 48$); Sydney, Australia ($n = 46$); and Strasbourg, France ($n = 48$). The lower limit of detection of the ELISA was 0.02 μg/m^2 (–·–) and samples below the detection limit are indicated (□).

or medium should be advised to consult their physician or allergist and consider avoidance procedures. These patients should also consider having the allergen concentration in their homes confirmed by ELISA. In most cases, negative or low test results would suggest that the level of exposure is not significant. Home characteristics questionnaires are a weak predictor of allergen levels in homes [34]. One of the goals of consumer-based testing is to educate patients about mite allergen levels and to provide objective information that patients can use in making decisions about exposure and about allergen avoidance [24, 35]. Allergists could also take such test results into account in their recommendations for treatment.

Our results clearly provide 'proof of principle' that lateral flow tests could be applied to other allergens, such as animal allergens, cockroach, foods and latex. We have developed prototype tests for cat (Fel d 1) and for peanut (Ara h 1) allergens and, in principle, the tests can be extended to any allergen for which suitable monoclonal antibodies are available. Further development of this technology will provide patients with a broad range of tests for environmental allergen exposure and will have industrial applications. These tests should improve the management of allergic disease by enabling patients to screen for environmental allergens and to take steps to reduce allergen exposure.

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