Rapid detection of fungal α-amylase in the work environment with a lateral flow immunoassay

Jelena Bogdanovic, MSc,[a,d] Marjo Koets, BSc,[b] Ingrid Sander, PhD,[c] Inge Wouters, PhD,[a,d] Tim Meijster, MSc,[d,e] Dick Heederik, PhD,[a,d] Aart van Amerongen, PhD,[b] and Gert Doekes, PhD[a] *Utrecht, Wageningen, and Zeist, The Netherlands, and Bochum, Germany*

Background: Occupational allergen exposure assessment usually requires airborne dust sampling at the worksite followed by dust extraction and enzyme immunoassay (EIA) analysis at the laboratory. Use of semiquantitative lateral flow immunoassays (LFIAs) may allow a more rapid detection procedure with direct on-site demonstration of a bioallergen exposure hazard.

Objective: In a field study, we evaluated a recently developed LFIA for fungal α-amylase, an important bakery allergen.

Methods: Airborne and surface dust (wipe) samples and samples from flours and baking additives used at the workplace were collected in 5 industrial bakeries and tested in the LFIA for fungal amylase. For comparison, amylase was measured in sample eluates with the reference EIA method.

Results: Sensitivity of the LFIA was 1 to 10 ng/mL, and of EIA, ~25 pg/mL. In LFIA, most flour samples, 84% of wipe samples, 26% of personal airborne dust, and none of the 26 ambient air dust samples produced a visible reaction. Wipe samples from dough-making areas and flour samples gave the strongest reactions. All extracts with >5 ng allergen per milliliter showed a positive LFIA reaction.

Conclusion: The LFIA for fungal amylase is an easy and rapid method to demonstrate the allergen directly at the worksite in less than 10 to 20 minutes. Similar LFIA methods may be used for other occupational allergens in other work environments.

Clinical implications: Lateral flow immunoassays for occupational allergens may be of great value in occupational hygiene surveys to demonstrate directly to workers and supervisors the hazards of work-related bioallergen exposure. (J Allergy Clin Immunol 2006;118:1157-63.)

Key words: *Allergen exposure, lateral flow immunoassay, fungal α-amylase, rapid detection of allergens*

Work-related exposure to bioallergens is a well known cause of occupational rhinitis and asthma. Exposure reduction measures based on systematic allergen monitoring can help to prevent or reduce the incidence of work-related allergies. Allergen measurement commonly includes airborne dust sampling at the workplace, and filter extraction and allergen-specific enzyme immunoassays (EIAs) at the laboratory. This procedure is labor-intensive, requires specialized equipment and laboratory facilities, and takes at least several days. Thus, there is a clear need for simpler methods for rapid identification and first characterization of a workplace hazard of bioallergen exposure—for example, as part of a routine workplace visit by industrial hygienists or other occupational health professionals.

Lateral flow immunoassay (LFIA) is a technique in which antigens are captured and concentrated between a line of immobilized specific antibodies on a nitrocellulose strip and another set of antigen-specific antibodies labelled with, for instance, gold or carbon particles, such that a colored line appears where the antigen is bound.[1-3] Test samples are first mixed with labeled antibody and applied to one end of the strip, after which the formed labeled immune complexes, driven by capillary forces, migrate through the nitrocellulose membrane to the line of immobilized capturing antibodies. Development of the reaction takes 5 to 30 minutes, depending on the antigen concentration in the sample. Because the method is rapid, portable, and user-friendly, the LFIA technique may be particularly suitable for semiquantitative allergen assessment in low-facility laboratories and field settings.

As part of the European Measurement of Occupational Allergen Exposure project, LFIAs with carbon-labeled detection antibodies have been developed for several bioallergens, including the bakery enzyme allergen fungal α-amylase.[4] In laboratory tests, these LFIAs detect the allergens with high specificity in solutions or filter extracts at levels as low as 1 to 10 ng/mL. In a field study, we investigated applicability of the newly developed amylase-LFIA for direct on-site demonstration of the allergen in

From [a]the Institute for Risk Assessment Sciences, Utrecht University; [b]the Agrotechnology and Food Sciences Group, Wageningen University and Research Centre; [c]Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin, Ruhr-University, Bochum; [d]Risk Assessment in the Work Environment, a collaborative center between the Institute for Risk Assessment Sciences (Utrecht) and the Dutch Organisation for Applied Scientific Research (TNO) Quality of Life (Zeist); and [e]the Business Unit, Food and Chemical Risk Analysis, TNO Quality of Life, Zeist.

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Reprint requests: Jelena Bogdanovic, MSc, Institute for Risk Assessment Sciences, Utrecht University, PO Box 80178, 3508 TD Utrecht, The Netherlands. E-mail: j.bogdanovic@iras.uu.nl.

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Abbreviations used
EIA: Enzyme immunoassay
GM: Geometric mean
LFIA: Lateral flow immunoassay
LOD: Limit of detection
PAS-6: Low-flow Personal Air Sampler for inhalable dust, with a 6-mm–wide orifice
PBT: PBS with 0.05% (vol/vol) Tween-20
PBTG: PBS with 0.05% (vol/vol) Tween-20 and 0.2% (wt/vol) gelatine
S&S: Schleicher & Schüll high-flow total dust sampler

samples from flours or other baking ingredients, and in surface dust from worksites with a presumed high or low level of amylase exposure. For comparison, we simultaneously performed conventional personal and stationary low-flow airborne sampling and stationary high-flow sampling of airborne dust, followed by extraction and measurement of amylase by specific EIA.

METHODS

The study was performed in 5 industrial bakeries (>20 workers per bakery) in The Netherlands. In each bakery, surface and airborne dust samples were taken in 2 main areas—dough-making and packing—with low and high flour dust exposure, respectively. Control filters were included in each sampling method. Samples were also collected from the materials the bakers worked with: basic baking flours and specific mixtures added as bread improvers to the flour during preparation of the dough, with known or suspected enzyme content.

Wipe sampling

Duplicate samples of settled dust were taken by wiping adjacent surfaces of approximately A4 paper size (20 cm × 30 cm) with filter papers (55 mm; Schleicher & Schuell, Dassel, Germany), prewetted with 250 to 300 μL PBS containing 0.05% (vol/vol) Tween-20 (PBT). In dough-making areas, samples were taken from the floor or the top of dough-mixing bowls, and in packing areas from the floor or the surface of the bread packaging line. In each bakery, 1 wipe sample was taken from a nonproduction area, such as the office or canteen, from a bookshelf or windowsill. Loaded and control filters were placed into 15-mL tubes (Greiner, Alphen aan den Rijn, The Netherlands), using clean gloves, and kept at +4°C until extraction the next day, or extracted and tested directly at the worksite (n = 5).

Sampling of airborne dust

Both stationary and personal sampling of airborne dust was performed, with sampling duration varying from 5 to 8 hours for all types of airborne samples. For stationary (ambient) sampling, the low-flow (2 L/min) inhalable dust sampler Personal Air Sampler with a 6-mm–wide orifice (PAS-6, University of Wageningen, Wageningen, The Netherlands)[5] and high-flow (23.5 L/min) Schleicher & Schüll total dust sampler (S&S, University of Wageningen)[6] were used, equipped with Teflon (PTFE) filters of 25 mm (Falp02500, Millipore Ireland, Cork, Ireland) and 47 mm (Falp04700, Millipore), respectively. Stationary PAS-6 samples were collected as duplicates and high-volume samples as single samples in dough-making and packing areas. Personal samples were obtained by using PAS-6 samplers. Sampled workers were bakers working in dough-making areas (n = 8), oven-bakers (n = 5), and baking assistants working in packing areas (n = 5). Other personal samples were collected from managers who performed various tasks, including preparation of dough (n = 2), from workers involved in technical maintenance of equipment (n = 2), and from a cleaner.

Elution of filters and flour samples

Teflon 25-mm filters were eluted by incubation for 60 minutes with 2.5 mL PBT in polystyrene tubes (5 mL; Greiner), with short (5 seconds) vortexing at 0, 30, and 60 minutes. After centrifugation at 3000*g*, supernatants were stored in aliquots at –20°C. The same procedure was used for 37-mm filters, with 5 mL PBT in 15-mL polypropylene tubes (Greiner).

Paper filters with wiped dust were eluted with 6 mL PBT in 15-mL polypropylene tubes (Greiner) by shaking by hand for a few seconds and vortexing for 10 seconds, and noncentrifuged eluates were stored in aliquots at −20°C. Centrifugation was not included because preliminary experiments had shown that both EIA and LFIA results were very similar for centrifuged and noncentrifuged eluates (data not shown), whereas a procedure without centrifugation would be much more convenient for routine use at the work site.

Flour samples were eluted as 5% (wt/vol) suspensions in PBT: 0.2 g flour or other baking ingredient was added to a 5-mL polystyrene tube (Greiner) and mixed with 4 mL PBT. The tube was vortexed at 0, 30, and 60 minutes and centrifuged at 3000*g*, and the supernatant was stored in small aliquots at −20°C.

Storage time for extracts did not exceed 1 month. A number (n = 5) of wipe extracts were tested in LFIA directly on site in the bakery immediately after allergen elution. Similarly, a number (n = 6) of flour samples were tested in the LFIA in a rapid procedure as used for the wipe samples: suspensions (teaspoon amount of flour in 4 mL PBT) were eluted by shaking by hand for 10 seconds, and the eluate tested without centrifugation directly in the LFIA. For comparison, another portion of the same eluate was centrifuged. Both portions were also analyzed by EIA.

Lateral flow immunoassay for fungal α-amylase

The LFIA for fungal α-amylase consists of a nitrocellulose strip with an impregnated affinity-purified rabbit antiamylase IgG (IRAS, Utrecht, The Netherlands)[7] as the capturing antibody, and a carbon-labeled mouse monoclonal antiamylase (clone IX3c4; BGFA, Bochum, Germany)[8] detecting antibody dried in a test tube.[4] The rabbit IgG antibody (500 ng/strip) was bound to the nitrocellulose (AE99; Whatman Schleicher and Schuell, Middelsex, United Kingdom) at 13 mm from the application end of the strip. At 18 mm, goat anti-mouse IgG was bound at 100 ng/strip to provide a positive control. Individual test strips and test tubes with dried detecting antibody were sealed in aluminium packages with silica desiccant pellets.

In the LFIA analysis (Fig 1), a test sample was mixed 1:1 with 2× concentrated running buffer (200 mmol/L borate buffer + 0.2% BSA + 0.2% Tween-20, pH 8.8), and 100 μL of the mixture was applied to the tube with carbon-labeled detecting antibody. After short and gentle mixing, during which the carbon-labeled antibody was allowed to dissolve and react with any amylase in the sample, the test strip with immobilized capturing antibody was inserted into the tube and reaction monitored for 60 minutes. Results, defined as appearance of a visible dark line at 13 mm from the base of the membrane, were read semiquantitatively (–, ±, +, ++) by the same observer 10, 30, and 60 minutes after application of sample, and were always compared with the results read by at least 1 additional observer. As allergen-specific control, we included a solution of 40 ng/mL fungal α-amylase (Fungamyl 1600S; Novo Nordisk, Bagsværd, Denmark) in each LFIA experiment, the same preparation as used for EIA calibration.

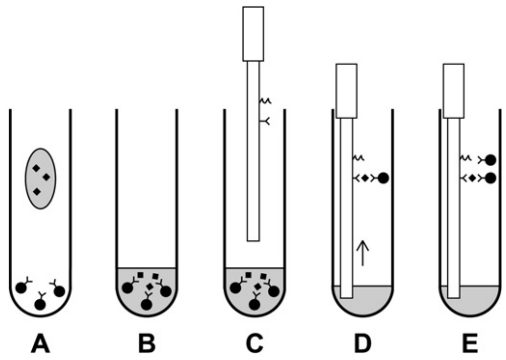

FIG 1. Lateral flow immunoassay for fungal α-amylase. **A,** Application of sample to the bottom of the tube with dried carbon-labeled antiamylase detecting antibody. **B,** Formation of immune complexes in the fluid phase. **C,** Addition of test strip to the tube. **D,** Migration of immune complexes through the strip, formation of complexes with the capturing antiamylase antibody on the membrane, and appearance of the test line. **E,** Appearance of the control line.

Enzyme immunoassay for fungal α-amylase

An EIA for fungal α-amylase with enhanced sensitivity was applied, using EIA amplification methodology[9] and the same monoclonal and polyclonal antibody reagents described. In validation experiments with airborne samples from the bakery environment, its limit of detection (LOD) was shown to be between 5 and 15 pg/mL for undiluted samples.

Microtiter plates were coated with 8 μg/mL mouse monoclonal IX3c4 antiamylase antibody, washed with PBT, and blocked with PBT containing 0.2% gelatine (PBTG). PBTG was used as dilution buffer in all assay steps except the last one (addition of substrate), and PBT as washing buffer between the steps. Fungal α-amylase standard (Fungamyl 1600S) was added at 6 serial dilutions (1 ng/mL to 31 pg/mL), and test samples at 1/2, 1/4, and 1/8 dilutions. Bound amylase was detected with biotinylated rabbit antiamylase IgG (1/500), followed by streptavidin-polyHRP (1/30,000; Research Diagnostics Inc, Flanders, NJ), and a 20-minute incubation with o-phenylenediamine (Sigma-Aldrich, Steinheim, Germany). After stopping the reaction with 2 mol/L HCl, the OD was read on 492 nm, and assay results were calculated from a 4-parameter best-fit calibration line (Softmax; Molecular Devices Corp, Menlo Park, Calif).

RESULTS

Analyzed samples from the 5 industrial bakeries included wipe samples of settled dust (n = 25), airborne stationary inhalable PAS-6 (n = 16), total dust S&S samples (n = 10), airborne personal PAS-6 dust samples (n = 23), and samples from flours and other bakery ingredients (n = 20). Dust levels on PAS-6 and S&S filters ranged from 0.02 to 18.2 mg. Amounts of wipe dust on paper filters were not measured but were estimated to be between 0.05 and 0.3 g on the basis of preliminary laboratory tests.

Allergen detection by LFIA

Allergen could be detected both in samples analyzed directly on site and in the extracts of samples analysed after storage at –20°C for as long as 1 month. A strong reaction with a distinct line at the site of the antiamylase capturing antibodies was observed on many strips on which a wipe or flour sample was applied. Several of the personal airborne dust samples also showed a detectable reaction (Fig 2). Positive reactions (+) were visible within maximally 30 minutes, and strongly positive (++) in less than 10 minutes. Approximately 15% of samples gave questionable (±) results, with very weakly stained test lines in LFIA, which were not detected by all observers, and are not or are hardly visible on photographic reproduction (Fig 2). Most of these samples (81%) were scored, however, as negative at 30 minutes, and as questionable only after 60 minutes. Interobserver scoring differences were maximally 1 grade on the semiquantitative scale, and positive reactions (+, ++) were always confirmed by all observers. Overall, the LFIA detected fungal α-amylase in 84% of wipe samples, in the majority of flour samples, and in 26% of personal airborne PAS-6 samples, whereas none of the stationary airborne dust samples (neither PAS-6 nor S&S) showed a positive reaction.

An overview of LFIA results for wipe and personal airborne PAS-6 samples is given in Table I. Allergen was found in wipe dust samples from both the dough-making and the packing area of the bakery, and from all types of surfaces: floor, mixing bowl, and packaging line. Duplicate samples from adjacent surfaces usually gave similar reactions in LFIA (Fig 2, *lanes 1-2 and 3-4*), but in 2 sets, large differences in reactivity were observed (not shown). Samples from the dough-making area gave mostly strong reactions (++), whereas samples from the packing area reacted more weakly (+), confirming different exposure levels (Fig 2, *lanes 1-4*). In one bakery, stronger reactions were found for wipe samples from the packing area than from the dough-making area, probably because in this bakery on the day of sampling, only dough for confectionary products was prepared, to which no amylase usually is added.[10] Control wipe samples taken from bookshelves or windowsills in nonproduction areas of bakeries, such as bakery office or canteen, were positive (+) in 3 out of 5 cases (Table I).

Lateral flow immunoassay–positive personal airborne samples were found among dough-makers, managers, oven-bakers, and workers involved in technical maintenance of equipment (Table I). Most of these samples had a much lower allergen content than wipe samples, although a few exceptions were found. The strongly positive personal sample shown in Fig 2 *(lane 10)* was obtained from a manager who performed several tasks during his work shift, like preparation of dough and cleaning of equipment with pressurized air.

The majority of samples from flours used in the bakery on the day of dust sampling were strongly positive (++) in LFIA (Fig 2, *lanes 20, 22, 23, 24*). Only a few samples of basic baking flours and flours used for confectionary products showed no reaction in the LFIA (Fig 2, *lane 21*). Among the field blanks—filter papers that had been prewetted at the worksite with PBT but not used for wiping (wipe blank; n = 5), or airborne dust filters mounted in

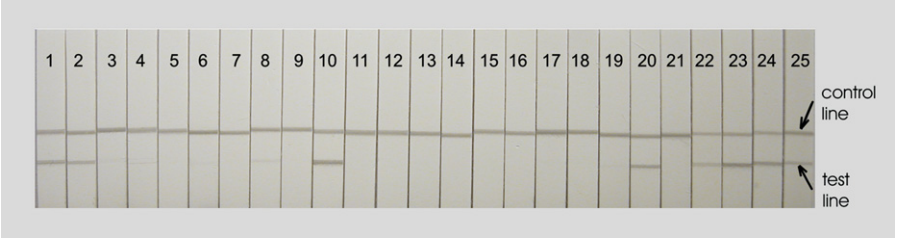

FIG 2. Lateral flow immunoassay analysis of samples from an industrial bakery. The analyzed samples were wipe samples from dough-making area *(1, 2)*, packing area *(3, 4)*, and bakery office *(5)*; airborne stationary PAS-6 *(7, 12, 13, 16)* and S&S samples *(18, 19)*; airborne personal PAS-6 samples *(8-10, 14, 15)*; flour samples *(20-24)*; field blanks *(6, 11, 17)*; and standard preparation of amylase allergen at 40 ng/mL *(25)*. Strongly positive *(++)* were samples 1 and 2 (wipes, dough-making area); 10 (personal airborne dust, manager); 20, 22, 23, 24 (flours); and 25 (standard). Positive *(+)* were samples 3 and 4 (wipes, packing area) and sample 8 (personal airborne dust, dough-maker). Questionable results *(±)*, with very weak staining of test line, were obtained with samples 6 and 17 (field blanks) and sample 19 (S&S). Other samples gave no visible reaction *(−)*.

TABLE I. Results of LFIA and EIA for fungal α-amylase, determined for wipe and personal PAS-6 samples from 5 industrial bakeries*

Wipe samples

		LFIA				EIA		
Sampling area	**N**	**N(–)**	**N(±)**	**N(+)**	**N(++)**	**Npos**	**GM (ng/mL)**	**Range (ng/mL)**
High exposure	10	0	2	3	5	10	33.8	0.9-687.2
Low exposure	10	0	0	10	0	10	1.1	0.2-21.3
Control (office, canteen)	5	2	0	3	0	5	0.6	0.1-3.1
Field blank	5	3	2	0	0	1	0.4	0.4

Personal PAS-6 samples

		LFIA				EIA		
Job title	**N**	**N(–)**	**N(±)**	**N(+)**	**N(++)**	**Npos**	**GM (ng/mL)**	**Range (ng/mL)**
Dough-maker	8	5	1	2	0	7	0.3	0.1-1.7
Baking assistant (packing)	5	4	1	0	0	0	—	—
Oven-baker	5	3	1	1	0	5	0.4	0.3-1.9
Manager (various tasks)	2	0	0	1	1	2	9.8	0.3-365.0
Technical maintenance	2	1	0	1	0	2	0.3	0.1-1.4
Cleaner	1	1	0	0	0	1	0.03	0.03

*LFIA results were graded as negative *(−)*, questionable *(±)*, positive *(+)*, and strongly positive *(++)*. EIA results are given as number of samples with detectable allergen levels *(Npos)*, together with GM and range of obtained allergen values for Npos.

a sampling head and removed without any air filtration (airborne blank; n = 9)—3 (21.4%) gave questionable (±) results, and the others remained completely negative.

Allergen quantification by EIA

The high sensitivity of the EIA resulted in a higher number of positive samples compared with the LFIA. Amylase was detectable in all wipe samples and 74% of the personal airborne PAS-6 samples (Table I), but in only 50% of stationary S&S and 12.5% of stationary PAS-6 samples (data not shown). Overall, the EIA results confirmed the LFIA findings: the highest amounts of amylase allergen were found in wipe samples from the dough-making area (geometric mean [GM] = 33.8 ng/mL) and much less in the packing area (GM = 1.1 ng/mL), whereas levels in extracts of personal (GM <1 ng/mL) and stationary airborne samples (GM <0.2 ng/mL) were much lower. Several personal airborne samples showed, however, remarkably high allergen concentrations—one of them even 365 ng/mL (at 18.2 mg dust load on the filter).

Most of the flour samples contained readily detectable amounts of α-amylase, in amounts of 52 ng/mL to 38.1 μg/mL, indicating an amylase content from approximately 1 to 762 mg enzyme per 1 kg flour. In EIA as well as in LFIA, extracts of flours that were analyzed with and without centrifugation showed comparable results.

The marked differences in levels, suggested by the LFIA for some duplicate wipe samples from adjacent surfaces, were confirmed by the EIA, showing 1.6-fold to 20-fold differences in allergen content. Thus, the distribution of amylase allergen in settled dust may be highly inhomogeneous. A similar observation was made for duplicate airborne stationary PAS-6 samples. All wipe samples from control areas in the bakery and 1 field blank (wipe) were positive for amylase allergen, but at relatively

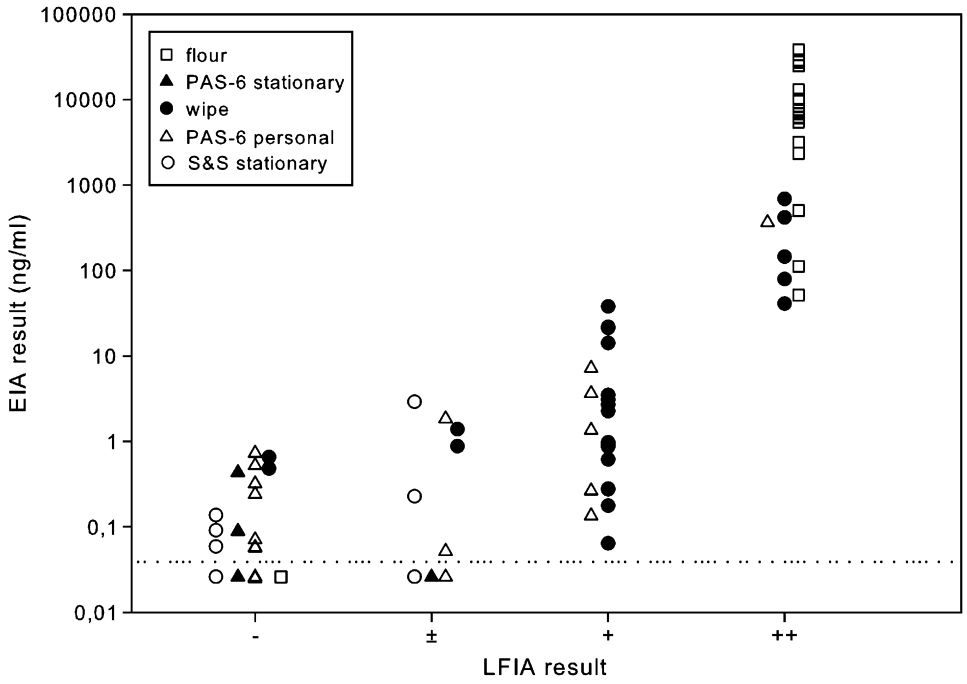

FIG 3. Agreement between LFIA and EIA results. LFIA results are given as negative *(−)*, questionable *(±)*, positive *(+)*, and strongly positive *(++)*. *Horizontal line* represents the LOD of the EIA.

low levels (0.4 ng/mL for field blank and 0.1-3.1 ng/mL for wipe controls).

Comparison of the 2 methods, LFIA and EIA, revealed a 100% agreement at allergen levels >5 ng/mL. Test samples with LFIA results classified as questionable (±) appeared in EIA to have allergen amounts <5 ng/mL or remained completely under the limit of detection of the EIA (Fig 3). Strongly positive (++) samples had allergen amounts > 30 to 40 ng/mL, whereas positive (+) samples had levels between 0.06 ng/mL and 30 ng/mL.

DISCUSSION

Assessment of exposure to allergens in work, home, or public environments usually involves laborious and time-consuming sampling, elution, and EIA measurement methods. There is a need for rapid measurement methods, which can demonstrate allergen presence directly on site. The case of fungal α-amylase exposure in bakeries is highly illustrative in this regard: it is a well known sensitizer to which as many as 10% to 20% of bakery workers may produce specific IgE,[11-13] but its airborne levels at the worksite are usually very low.[7] Few laboratories have immunoassay methods available for its detection at nanogram or even picogram levels,[7,8,14,15] but the procedure can not be completed within less than a few days. Hazard identification may in principle be achieved by making an inventory of products and ingredients used in the bakery. However, bakery enzymes like fungal amylase are usually present in mixtures of additives or batches of enriched flours without clear labeling. Under such circumstances, a test that rapidly detects and gives a first semiquantitative estimation of levels of the allergenic enzyme in flours or settled dust from the worksite may be helpful.

Lateral flow technology has been introduced and validated in veterinary, feed and food, and human sample analysis (eg, HIV,[16,17] influenza,[18] or respiratory syncytial virus[19] tests). Recently it has found its application for rapid detection of mite allergens in the homes,[1,20] and was shown to be very sensitive and well correlated with results of the reference EIA methods. Our studies would be the first to explore systematically the applicability of a similar methodology for the detection of occupational allergens at the worksite. We used an LFIA developed as part of a collaborative European Union project, in which we combined the LFIA expertise of 1 partner with use of antiamylase antibodies from 2 other partners.[4] The presented results show that this rapid semiquantitative technique indeed may be used by occupational health professionals in bakeries and flour mills. Sampling and elution methods are short and simple, and suitable for rapid assay performance. As shown by preliminary experiments, wipe or flour samples can be eluted in PBT by simple manual shaking for 10 seconds and no centrifugation, without interfering with the assay performance. With the LFIA, an occupational hygienist can provide the bakery workers with information on a potential health risk at the worksite in less than 30 minutes. For example, identification of amylase-containing flours by LFIA allows sensitized bakers to avoid handling of these flours or to use better personal protective measures.

We validated the LFIA for fungal α-amylase in 5 industrial bakeries and compared it with the reference quantitative EIA. Although less sensitive than the EIA, the LFIA technique appeared sufficiently sensitive (LOD = 1-10 ng/mL) for detecting amylase at moderate or high levels in airborne dust samples. However, because levels below 1 ng/m^3 have also been shown to be potentially sensitizing,[10] the technique would generally not be suitable for allergen detection in airborne samples obtained with conventional air flow and sampling duration. Compared with airborne dust samples, samples of surface dust or enzyme-containing flours showed much higher and easily detectable amylase levels in LFIA. Thus, during surveys, LFIA was able to detect allergen within 10 minutes for strongly positive (++) and within 20 to 30 minutes for moderately positive (+) samples. LFIA analyses of various flours used in the bakeries on the day of sampling showed that most batches were potential sources of airborne amylase allergen. The allergen was also demonstrated in the majority of wipe extracts (84%) and in 26% of personal airborne dust samples. The lower detection limit for personal airborne samples appeared to be 5 ng/m^3 (ie, 2 ng/mL extract).

Three out of 5 wipe samples from nonproduction areas (bakery office, canteen) were positive in LFIA, and all of them were positive in EIA. On the other hand, none of the stationary airborne samples (low or high flow) from production areas was positive in LFIA. This demonstrates the advantage of wipe sampling compared with airborne (particularly stationary) sampling, which often fails to capture the allergen, whereas high sensitization rates among workers indicate that there must be moments of exposure to the allergen at levels that with available immunoassays should be detectable.[21] An explanation for these findings, contradictory at first sight, might be a marked heterogeneity and variation in time and space of amylase-bearing airborne particles, as confirmed by the large differences in EIA-determined allergen contents in both duplicate airborne and wipe samples. Capturing of allergenic amylase particles (approximately 1 per 100,000 flour dust particles) by airborne sampling would be often unsuccessful. Therefore, to avoid false-negative sampling results, taking duplicate or even series of repeated airborne or settled dust samples from the same sampling site may be recommended. Highly variable levels of exposure may be even more likely in low exposure sites in the bakeries, for instance, because of incidental spillage of flours. Amylase-positive samples in wipes from nonproduction areas stress the importance of monitoring occupational exposure and the necessity for strict exposure reduction measures, not only in production but also at worksites that a priori might have been regarded as allergen-free.

Lateral flow immunoassay results were well correlated with those of the EIA, and all samples with amylase levels >5 ng/mL in EIA gave a positive or strongly positive LFIA reaction. At lower allergen levels, some discrepancies between the 2 methods were observed because of a lower sensitivity of the LFIA and possibly to weak nonspecific reactions in LFIA. Also, difficulties in clearly distinguishing positive from negative results because of a very weak staining of LFIA test line (±) were observed at these levels. Vague LFIA lines mostly appeared only after 30 to 60 minutes of reaction, indicating that for practical use, 30 minutes should be the maximal reading time, in accordance with recommendations for many similar commercial 1-step assays. Besides, allergen levels <10 ng/mL were mainly found in airborne samples, whereas amylase LFIA is primarily suitable for analyses of wipe or flour samples, where allergen levels are considerably higher.

In conclusion, LFA was shown to be applicable for rapid detection of fungal α-amylase allergen in bakeries and flour mills. By analyzing wipe or flour samples, the technique provides an opportunity for quick investigation and direct on-site demonstration of allergens in the work environment. Our results suggest that the LFIA technique may be also applied for rapid on-site detection of other occupational allergens, such as rat and mouse urinary proteins in laboratory animal facilities.

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