

Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: Relations with culturable fungi, reported home dampness, and respiratory symptoms

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Background: Epidemiologic studies have demonstrated an association between indoor fungal growth and respiratory symptoms. However, in only a few studies was fungal exposure actually measured.

Objective: The purpose of this study was to evaluate the measurement by enzyme immunoassay of extracellular polysaccharides of *Aspergillus* and *Penicillium* species (EPS-Asp/Pen) in house dust as a marker for fungal exposure and to study the relations between EPS-Asp/Pen levels and home dampness and respiratory symptoms in children.

Methods: Extracts of house dust samples from bedroom and living room floors and mattresses from homes of 31 children with chronic respiratory symptoms and 29 children with no chronic respiratory symptoms were analyzed for EPS-Asp/Pen.

Results: EPS-Asp/Pen were readily detectable (40 to 46,513 nanogram equivalent/g dust) in 161 house dust extracts, with highest concentrations in living room floor dust. EPS-Asp/Pen levels were 2 to 3 times higher on carpeted floors than on smooth floors. EPS-Asp/Pen were significantly correlated with total culturable fungi ($r = 0.3$ to 0.5) and with house dust mite allergens ($r = 0.3$ to 0.5). EPS-Asp/Pen levels in living room floor dust were positively associated with occupant-reported home dampness. This was not observed for EPS-Asp/Pen in bedroom floor and mattress dust. EPS-Asp/Pen levels in living room floor dust were positively associated with respiratory symptoms. EPS-Asp/Pen in bedroom floor and mattress dust showed a reversed association with respiratory symptoms, possibly because of allergen-avoidance measures taken in the bedroom.

Conclusion: The enzyme immunoassay for fungal EPS-Asp/Pen

may be a useful method for exposure assessment of indoor fungi. (*J Allergy Clin Immunol* 1999;103:494-500.)

Key words: Fungi, house dust, extracellular polysaccharides, home dampness, respiratory symptoms

Exposure to mite and pet allergens in the home environment is an important risk factor for the development of respiratory allergy and asthma. The role of fungal exposure is less well understood. Several population-based studies have suggested that fungal exposure, together with house dust mite exposure, might account for the frequently reported association between living in damp housing conditions and respiratory disorders.¹⁻⁵ Exposure to fungi and damp housing conditions in these studies has invariably been assessed by questionnaire, however. Although several authors have attempted to relate reports of dampness and exposure to fungi, it is currently not clear to what extent questionnaire reports of damp and mold spots correlate with exposure to relevant fungal components. It is also not clear which fungal components and which inflammatory and/or allergic mechanisms primarily account for the presumed pathogenic effects of indoor fungal exposure. Atopic sensitization to fungal allergens may play a role,^{5,6} but other inflammatory mechanisms might also be of importance, including type III allergy to fungal-specific antigens or nonimmune inflammatory reactions to fungal components (eg, $\beta[1 \rightarrow 3]$ -glucans, a major cell wall component of fungi).⁷⁻⁹

Limited data from field studies are available on indoor fungi and fungal allergen levels. The traditional counting of culturable propagules in house dust has been applied in some studies¹⁰⁻¹² but has serious drawbacks, including poor reproducibility and the fact that only culturable organisms are counted. Quantitative assessment of major fungal allergens in house dust or indoor air has been reported only incidentally. Few major fungal allergens have been characterized, and allergen stability and production may be highly variable.¹³⁻¹⁵ General markers for fungal biomass in house dust or indoor air, such as ergos-

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Abbreviation used

CFU:	Colony-forming unit
EIA:	Enzyme immunoassay
EPS-Asp/Pen:	Extracellular polysaccharides of <i>Aspergillus</i> and <i>Penicillium</i> species
GM:	Geometric mean
GSD:	Geometric standard deviation
ng eq:	Nanogram equivalent
PBT:	PBS containing 0.05% (vol/vol) Tween-20
PBTB:	BSA in PBT

terol and $\beta(1\rightarrow3)$ -glucans, have been described¹⁶⁻¹⁸ but have not yet been routinely applied in indoor studies and do not allow identification of fungal species or genera.

There is therefore a need for specific, sensitive, and cost-efficient methods to assess fungal exposure quantitatively in the indoor environment that would allow at least partial identification of fungal species present. In this study a sandwich enzyme immunoassay (EIA) was evaluated, with which extracellular polysaccharides (EPS) of *Aspergillus* and *Penicillium* species (EPS-Asp/Pen) can be specifically measured. EPS are stable carbohydrates secreted or shed during fungal growth, with an antigenic specificity usually at the genus level, although EPS-Asp/Pen are cross reactive.¹⁹⁻²² At present, there is no evidence for a pathogenic role of EPS in allergic or inflammatory reactions to fungal components. However, EPS may be a good quantitative marker for fungal biomass in house dust. In addition, genus-specific EPS assays may provide important qualitative information allowing differentiation between indoor and outdoor fungal genera. We report EPS-Asp/Pen levels in house dust in a sample of 60 homes in which dust samples had been taken for mite allergen measurements and for the determination of culturable fungi in a previous study.^{11,23} The relationship with culturable fungi was investigated, and relationships between EPS concentrations and signs of mold and dampness in the home were studied. Moreover the relationship between EPS-Asp/Pen and respiratory symptoms in children living in these homes was studied.

METHODS

Study population

Samples of settled house dust were obtained in October and November 1990 from 60 homes in The Netherlands. The homes were selected from a group of 516 homes that were included in a case-control study on dust mite allergen exposure and respiratory symptoms in children (aged 6 to 12 years).²⁴ The selection has been described previously.¹¹ Briefly, homes were selected on the presence or absence of (1) mold or damp spots anywhere in the home of the child, observed by the investigators during a home visit; and (2) reported respiratory symptoms (chronic wheeze, chronic cough, attacks of shortness of breath with wheezing, or doctor-diagnosed asthma or bronchitis) of children living in those homes. Thus 4 groups of homes were selected: (1) homes of children without respiratory symptoms and without observed dampness ($n = 13$); (2) homes of children without respiratory symptoms but with observed dampness ($n = 16$); (3) homes of children with respiratory symptoms, without observed dampness ($n = 12$); and (4) homes of children with respiratory symptoms, with

observed signs of dampness ($n = 19$). Two subgroups of symptomatic subjects were defined (ie, subjects with doctor-diagnosed asthma [asthma; $n = 9$] and subjects with doctor-diagnosed bronchitis [bronchitis; $n = 9$]). Characteristics of the study population have been described elsewhere.¹¹ Respiratory symptoms and resident-reported dampness were assessed by questionnaire.^{11,23}

Dust sampling and extraction

Dust was sampled from floors of living rooms and bedrooms (2 m²) and from the childrens' mattresses, according to an internationally standardized protocol²⁵ as described previously.²³ When rooms had a smooth floor with a rug, 1 m² of the rug and 1 m² of the smooth floor cover adjacent to the rug were sampled. Dust samples were stored at -20°C until extraction. Of each dust sample, 200 mg was extracted after being shaken in 2 mL ammonium bicarbonate buffer (0.125 mol) with NaN_3 (0.1% wt/vol) for 2 hours.²³ Extracts were stored at -20°C before analyses of house dust mite allergen (Der p I) and EPS-Asp/Pen content. Der p I was determined as previously described.²³ In total 19 extracts, of which 18 were from living room floor dust, were not available for EPS-Asp/Pen analysis because the entire volume was used for mite allergen analyses.

Separate dust samples were collected in the same time period from bedroom floors and mattresses of the same homes to determine the concentration of culturable fungi in dust.¹¹

EPS-Asp/Pen production

EPS from *P digitatum* was prepared as previously described.¹⁹ Briefly, spores of *P digitatum* were inoculated in dialyzed malt extract broth and incubated at 25°C for 7 days with orbital shaking at 90 rpm. NaN_3 was added (0.1% wt/vol), and incubation continued for another 16 hours at 25°C . Mycelia and proteins were removed by filtration and ammonium sulfate precipitation, respectively. The supernatant was dialyzed against distilled water and applied to a PBS-equilibrated Sepharose CL-4B gel filtration column (2.5×95 cm). Column fractions were tested by an EPS-Asp/Pen EIA; and peak fractions were pooled, concentrated with Amicon concentrators (Amicon Inc, Beverly, Mass) dialyzed against distilled water, and freeze dried. The antigenic responses against EPS from *P digitatum* are directed mainly to $\beta(1\rightarrow5)$ -D-galactofuranosides.²⁶ The antigenic properties of EPS are identical for all species within the *Penicillium* and *Aspergillus* genera. Anti-EPS-Asp/Pen IgG antibodies were raised in rabbits of which a part was conjugated with peroxidase.¹⁹

EPS-Asp/Pen analysis

EPS-Asp/Pen were measured in dust extracts 4 years after sample collection, with a sandwich EIA. An essentially identical assay was described earlier as a means for screening for fungal contamination of food products.^{19,27} Polystyrene high-capacity microtiter plates (no 655061; Greiner, Nuertinger, Germany) were coated overnight at 4°C with polyclonal rabbit anti-EPS-Asp/Pen IgG antibodies (100 μL of 10 $\mu\text{g}/\text{mL}$ antibody in PBS; pH 7.0). After being washed with PBS containing 0.05% (vol/vol) Tween-20 (PBT), 100 μL of 5 serial dilutions (diluted in 1% wt/vol BSA in PBT [PBTB]) of test samples (routinely tested at 1/10 to 1/160) and 10 serial dilutions of EPS standard (0.05 to 25 ng/mL) were added to the wells. For calibration, EPS of *P digitatum* was used. Each plate contained 10 negative controls (blanks; PBTB). Samples were incubated at 22°C for 1.5 hours. After being washed, 100 μL of peroxidase-labeled polyclonal rabbit anti-EPS-Asp/Pen IgG antibodies diluted 1/2000 in PBTB was added and incubated for 1.5 hours at 22°C . After being washed, 200 μL tetramethylbenzidine (0.1 mg/mL) in 0.11 mol citric acid/acetate buffer, pH 5.5, containing 0.006% H_2O_2 was added and incubated for 20 minutes at 20°C . The enzyme reaction was terminated with 50 μL 2 N H_2SO_4 , and optical density was read at 450 nm (OD-450).

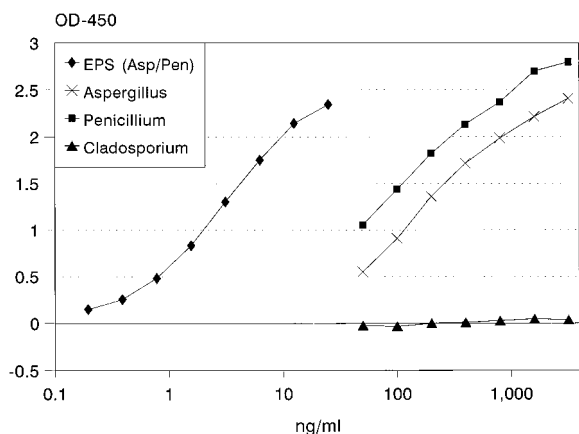


FIG 1. Dose-response curves for 3 fungal mixtures (*Aspergillus* spp, *Penicillium* spp, and *Cladosporium* spp) and the EPS reference preparation (*P digitatum*) in the EPS-Asp/Pen EIA.

Because, at lower dilutions, dose-response curves of the samples were often not parallel to that of the standard, the EPS-Asp/Pen concentrations were estimated by end-point titration, comparing the titer of each extract with the titer of the standard. The titer was defined as the dilution at which an OD-450 of 0.1 above the background of the reagent blank was found. To calculate the titer, a regression was performed between log dilution and logit transformed OD-450 values of the multiple diluted standard or samples. The EPS-Asp/Pen concentration in a test sample was calculated as the ratio between the titer of the sample and the titer of the standard multiplied by the EPS concentration of the standard. Because not all EPS standard preparations show, on a weight basis, equal reactivity in the EIA, sample concentrations were expressed in nanogram-equivalents per gram (ng eq/g) of dust, thus referring to the concentration of the EPS standard preparation used in this particular study. All samples were analyzed in duplicate in 2 independent test runs, and concentrations were presented as the mean of the duplicate analyses.

Crude fungal extracts

Mixtures of crude extracts of 4 *Aspergillus* species (*A versicolor*, *A fumigatus*, *A amstelodami*, and *A nidulans* [prod no 22.00; ALK Benelux, Houten, The Netherlands]), of 4 *Penicillium* species (*P brevicompactum*, *P expansum*, *P notatum*, and *P commune* [prod no 25.00; ALK]), and of 3 *Cladosporium* species (*C cladosporioides*, *C elatum*, and *C herbarum* [prod no 23.00; ALK]), respectively, were used to confirm the specificity of the EPS-Asp/Pen EIA.

Measurement of indoor relative humidity

In the child's bedroom of 19 dwellings, relative humidity and temperature were measured for periods of 22 to 42 days in the same time period as dust sampling was performed.²³ The average relative humidity for the whole measurement period was used in further analyses.

Statistical analysis

Statistical analyses were performed with SAS (version 6.12; SAS Institute, Cary, NC). Because data followed a normal logarithm distribution, EPS-Asp/Pen concentrations were normal logarithm transformed, and mean concentrations were expressed as geometric means (GM) with geometric standard deviations (GSD). Pearson correlations were calculated. The association between EPS-Asp/Pen levels and colony-forming unit (CFU) counts was assessed by multiple regression analysis on normal logarithm-transformed data. Statistical testing comparing EPS-Asp/Pen levels in homes with and without specific characteristics (type of floor, damp and mold spots)

TABLE I. GM and GSD of EPS-Asp/Pen concentrations in nanogram equivalents per gram of house dust from living room and bedroom floors and mattresses; textile floor cover was compared with smooth floor cover

Location	n	GM	GSD	Range (min-max)
Living room	42	2919	4.2	131-46513
Smooth	5	952	3.6	253-3630
Textile	37	3395*	4.0	131-46513
Bedroom	60	987	3.9	40-21917
Smooth	18	530	2.8	78-3496
Textile	42	1289†	4.1	40-21918
Mattress	59	1281	2.8	93-27806

* $P < .1$.

† $P < .05$; compared with smooth floor covers.

and comparing EPS-Asp/Pen levels between nonsymptomatic and symptomatic subjects was performed with a *t* test on normal logarithm-transformed data. When differences between floors with different covers were considered, smooth floors with a rug were grouped together with carpet floor covers. Odds ratios (ORs) were calculated by means of logistic regression. The continuous EPS-Asp/Pen exposure variables were dichotomized with median values as cutpoints (living room, 2495 ng eq/g; bedroom, 957 ng eq/g; mattress, 1530 ng eq/g) to define high and low exposure groups and to calculate ORs for various health outcome variables. All ORs were adjusted for mite allergen levels. Der p I exposure variables were therefore dichotomized on the basis of their median values (living room, 7.2 µg/g; bedroom, 2.4 µg/g; mattress, 4.1 µg/g).

RESULTS

The detection limit of the assay was approximately 0.2 ng eq/mL. Reproducibility expressed as a coefficient of variation was 16% on the basis of duplicate analyses. Commercial crude fungal extracts of *Aspergillus* and *Penicillium* species showed dose-response curves parallel to that of the EPS-*P digitatum* standard at concentrations ranging from 50 to 5000 ng/mL. In contrast, the extract of the *Cladosporium* species mixture showed no response at these concentrations (Fig 1). The same *Cladosporium* preparation contained comparable amounts of *Cladosporium*-specific EPS, as determined with a similar EIA with specific rabbit polyclonal anti-*Cladosporium*-EPS antibodies (data not shown).

EPS-Asp/Pen concentrations were readily detectable in the 161 available house dust extracts (Table I). EPS-Asp/Pen concentrations were approximately 2 to 3 times higher in house dust from textile floor covers compared to smooth floors. In living room floor dust, EPS-Asp/Pen concentrations were highest, almost 4 times higher than EPS-Asp/Pen concentrations in bedroom floor dust samples (paired comparison of data from textile floor covers only; $P < .01$; $n = 37$).

EPS-Asp/Pen concentrations in bedroom floor and mattress dust were significantly but not strongly correlated with total culturable fungi and fungi of various specific genera (Table II). All of the individual genus-specific fungal counts were significantly correlated with total CFU counts ($r = 0.4$ to 0.8 ; $P < .01$), with the excep-

tion of *Ulocladium* species in floor dust samples, and *Aspergillus* species in mattress dust samples (data not shown). In bivariate analyses with total culturable fungi and individual genus-specific fungal counts in one regression model, EPS-*Asp/Pen* levels appeared to correlate in all cases more strongly with counts of total fungi than with counts of any of the individual genera, including *Aspergillus* + *Penicillium* (data not shown).

EPS-*Asp/Pen* levels were significantly correlated with Der p I levels in living room floor, bedroom floor, and mattress dust, with correlation coefficients of 0.39, 0.47 and 0.32, respectively ($P < .05$). Similar to what was observed for EPS-*Asp/Pen* levels, highest Der p I levels were measured in living room floor dust (GM = 4228 ng/g) compared with bedroom floor dust (GM = 2099 ng/g).

Table III compares EPS-*Asp/Pen* levels in house dust between homes with occupant-reported damp and/or mold spots in the living room, bedroom, or anywhere in the home and homes with no damp spots or mold spots in the home. The analyses with floor dust EPS-*Asp/Pen* concentrations only included observations from textile floor covers, to avoid confounding by differences in EPS-*Asp/Pen* concentrations between textile and smooth floor covers. EPS-*Asp/Pen* levels were approximately 6 and 4 times higher ($P < .01$) in living rooms with reported mold and damp spots, respectively, compared with living rooms in homes where no mold or damp spots were reported. No clear associations were found for bedroom and mattress EPS-*Asp/Pen* concentrations between bedrooms with reported damp and mold spots and bedrooms from homes without damp or mold spots. EPS-*Asp/Pen* concentrations were 2 to 3 times higher in living rooms and only slightly higher in bedrooms and mattresses in homes with reported damp and mold spots (anywhere in the home) compared with homes with no damp and mold spots. No relation between EPS-*Asp/Pen* concentrations and investigator-observed damp and mold spots was found (data not shown). Mattress EPS-*Asp/Pen* concentrations were correlated with relative humidity of the air in the bedroom ($R = 0.54$; $P < .05$), whereas no correlation with EPS-*Asp/Pen* concentrations in bedroom floor dust was found.

EPS-*Asp/Pen* levels in the living room were higher in homes of symptomatic children compared with nonsymptomatic children (control subjects), whereas they were lower in bedroom floor and mattress dust samples (Table IV). Consequently, ORs showed positive associations between living room floor EPS-*Asp/Pen* concentration and respiratory symptoms, with highest OR for doctor-diagnosed asthma (OR = 9.5; $P < .1$), and negative associations for bedroom and mattress EPS-*Asp/Pen* concentrations. All ORs were adjusted for Der p I levels. Confidence intervals for ORs associated with living room EPS-*Asp/Pen* concentrations were wide for the asthma and bronchitis group because of the small number of children with these symptoms and the relatively small number of control children for whom living room exposure data were available. A reversed trend for EPS-*Asp/Pen* levels of bedroom floors and mattresses was possibly due to

TABLE II. Pearson correlation coefficients for the relation between EPS-*Asp/Pen* concentrations (nanogram equivalents per gram of dust) and CFU concentrations (CFUs per gram of dust) of the most frequently isolated fungal species and total number of culturable fungi

Fungal species	Bedroom	Mattress
<i>Aspergillus</i> spp	0.22 (26/36)	0.02 (30/37)
<i>Penicillium</i> spp	0.36 (36/36)†	0.14 (34/37)
<i>Eurotium</i> spp	0.41 (21/36)†	0.20 (29/37)
<i>Cladosporium</i> spp	0.39 (36/36)†	0.28 (34/37)*
<i>Alternaria</i> spp	0.46 (31/34)‡	0.25 (37/37)
<i>Ulocladium</i> spp	0.26 (23/35)	0.06 (32/37)
<i>Aspergillus</i> + <i>Penicillium</i> spp	0.37 (36/36)†	0.20 (37/37)
Total CFU	0.45 (53/53)‡	0.34 (55)†

The number in parentheses denotes the detectable CFU counts per total number of observations.

* $P < .1$.

† $P < .05$.

‡ $P < .01$.

allergen avoidance measures taken particularly in the child's bedroom. Thirty percent of the parents of symptomatic children had purchased a new floor cover in the child's bedroom because of respiratory symptoms of their children, whereas none of the parents of control subjects had done so. These differences in allergen avoidance measures between patients and control subjects were much smaller when new floor covers in the living room were considered (16% vs 7%). Moreover, 10% of the patients had changed mattresses because of respiratory complaints compared with 0% of the control subjects. Restricting the analyses to bedrooms where no allergen avoidance measures had been taken and no smooth floor covers were present resulted in marginally higher and less significant ORs for general respiratory symptoms. For bedroom floors, the OR shifted from 0.2 ($P < .01$) to 0.3 (95% CI, 0.1 to 1.1; $P < .1$; $n = 39$, of which 18 were symptomatic), and for mattresses, the OR shifted from 0.3 ($P < .05$) to 0.5 (95% CI, 0.4 to 5.0; $P > .1$; $n = 38$, of which 18 were symptomatic). A similar statistical analysis, adjusting for smooth floor covers and allergen avoidance measures, could not be performed for the individual asthma and bronchitis groups because of the low number of subjects with these symptoms.

DISCUSSION

In this study we evaluated a sandwich EIA to measure EPS-*Asp/Pen* as a marker for indoor fungal exposure. EPS-*Asp/Pen* could readily be measured with adequate reproducibility and sensitivity in settled house dust. Main advantages of EPS tests are the ability to, at least partially, identify specific fungal genera, store samples frozen before analysis, and maintain low costs per sample.

EPS-*Asp/Pen* levels were significantly, but not strongly, correlated with counts of culturable fungi. Reasons may be (1) that only culturable fungi were counted, although EPS-*Asp/Pen* most likely represents total fungal

TABLE III. Association of EPS-*Asp/Pen* concentrations (nanogram equivalents per gram of dust) and self-reported mold and damp spots in the living room, bedroom, or anywhere in the home

Mold and damp patches	Living room		Bedroom		Mattress	
	n	GM	n	GM	n	GM
No mold or damp spots in the home	14	2077 (2.9)	17	1150 (3.3)	21	1080 (2.9)
Mold spots in the room	4	12875 (1.5)‡	2	568 (—)	3	590 (3.1)
Damp spots in the room	10	8594 (3.7)‡	4	810 (1.8)	7	1016 (2.3)
Mold spots in the home	20	4235 (4.4)	17	2038 (3.9)	28	1478 (2.9)
Damp spots in the home	20	5843 (4.0)†	21	1251 (4.3)	31	1496 (2.7)
Mold or damp spots in the home	23	4580 (4.4)*	23	1491 (4.6)	35	1430 (2.7)

GM values shown with GSD.

Samples from smooth floor covers were excluded from the analyses.

* $P < .1$; compared with homes with no mold or damp spots.† $P < .05$; compared with homes with no mold or damp spots.‡ $P < .01$; compared with homes with no mold or damp spots.**TABLE IV.** EPS-*Asp/Pen* concentrations (GM) and ORs for high and low EPS-*Asp/Pen* concentrations, comparing the nonsymptomatic subjects with subjects with general respiratory symptoms, doctor-diagnosed asthma, and doctor-diagnosed bronchitis

	No respiratory symptoms	Respiratory symptoms	Asthma	Bronchitis
Living room (n)	18	24	7	8
EPS- <i>Asp/Pen</i> (ng eq/g)	2568 (3.8)	3214 (4.5)	7709 (3.5)*	3815 (3.2)
OR	1.0	2.5 (0.7-9.3)	9.5 (0.9-103.5)*	3.3 (0.5-20.9)
Bedroom (n)	29	31	9	9
EPS- <i>Asp/Pen</i> (ng eq/g)	1549 (4.1)	647 (3.3)†	781 (4.1)	386 (3.7)†
OR	1.0	0.2 (0.0-0.6)‡	0.1 (0.0-0.7)†	0.2 (0.0-1.1)*
Mattress (n)	28	31	9	9
EPS- <i>Asp/Pen</i> (ng eq/g)	1771 (2.3)	956 (2.9)†	1765 (2.4)	521 (3.3)‡
OR	1.0	0.3 (0.1-0.9)†	0.8 (0.2-3.9)	0.2 (0.0-0.9)†

GSD and 95% confidence intervals are shown in parentheses.

Analyses were controlled for Der p I concentrations.

* $P < .1$; compared with the nonsymptomatic group.† $P < .05$; compared with the nonsymptomatic group.‡ $P < .01$; compared with the nonsymptomatic group.

biomass of *Aspergillus* and *Penicillium* species, including nonculturable spores and mycelia; and (2) that CFU and EPS-*Asp/Pen* measurements were performed on 2 different dust samples. We previously reported that, in the same study sample, a poor reproducibility of total CFU counts in duplicate house dust samples (taken within a 6-week interval) from the same location was found.²⁸ Variability in isolated genera and species between duplicate samples was even more substantial. Measurement error for genus-specific culturable counts was thus larger than for total culturable counts. Considering that *Aspergillus* and *Penicillium* species constitute a large and relatively constant fraction of total culturable fungi, it is likely that the difference in measurement error may explain why EPS-*Asp/Pen* levels in house dust were more strongly associated with total culturable counts than with genus-specific counts of *Penicillium* and *Aspergillus*. A high correlation between the sum of *Aspergillus* and *Penicillium* species and total culturable fungi ($r = 0.8$ for bedroom floor and 0.5 for mattresses; $P < .01$) indicated that the fraction of *Aspergillus* and *Penicillium* species was indeed more or less constant. A con-

sistent significant association with total culturable fungi suggests that, at least in this study, EPS-*Asp/Pen* was a good marker for total fungal biomass. Whether this is generalizable to other indoor studies is unclear and probably depends on the variation and rank order of the genera present in the environment of those studies.

Significant associations between EPS-*Asp/Pen* and the other culturable fungal genera were most likely due to correlation between total CFUs and CFU counts of specific genera. Cross-reacting epitopes of EPS from other genera with the anti-EPS-*Asp/Pen* antibodies do not seem likely, because a crude extract of 3 *Cladosporium* species was not reactive in our assay (Fig 1). Moreover, the specificity of the anti-EPS-*Asp/Pen* antibodies has previously been confirmed in a sandwich assay with 32 fungal species, in which none of the following genera showed positive reactions: *Mucor*, *Rhizopus*, *Botrytis*, *Geotrichum*, *Cladosporium*, *Fusarium*, and *Tricothecium*.²⁰

Smooth floor covers contained 2 to 3 times less EPS-*Asp/Pen* per gram of dust compared with textile floor covers. Fungal CFU counts in bedroom floor samples showed a similar difference.¹¹ Wickman et al¹² reported

only slightly higher numbers of CFUs in dust from wall-to-wall carpets. A substantial difference in concentration (6 to 14 times) between smooth and textile floor covers is generally also observed for house dust mite allergens in The Netherlands.²³ Levels of EPS-Asp/Pen were higher in living room floor dust than in bedroom floor or mattress dust, similar to what was observed for Der p I levels. EPS-Asp/Pen levels were moderately correlated with levels of Der p I. Wood et al²⁹ also demonstrated an association between fungal CFU counts and dust mite allergens. The correlations are probably due to dependence of both mites and fungi on high humidity levels.

EPS-Asp/Pen levels in living room floor dust were higher when occupants reported mold or damp spots in the living room or anywhere in the home, whereas bedroom floor and mattress EPS-Asp/Pen levels did not show associations with a report of mold or damp spots in the bedroom or anywhere in the home. Mattress EPS-Asp/Pen concentrations were, however, correlated with relative air humidity in the bedroom. Lack of a clear association between EPS-Asp/Pen levels and damp characteristics with bedroom dust samples might have been due to allergen avoidance measures, taken particularly in the bedroom. This may also explain why we observed much lower levels of EPS-Asp/Pen in bedroom floor dust compared with living room floor dust. Several studies have investigated the relation between signs of fungal growth and dampness in the home and fungal CFU counts in house dust or in the air and have found at best only weak associations.^{1,11,12,17,30,31} A reason for this lack of a stronger association may be found in the limitations of the traditional methods to assess fungal exposure (CFU counts) in these studies, as discussed earlier in this paper, and by Dales et al.¹⁷ The relatively strong association found in our study for living room concentrations may indicate that the EPS-Asp/Pen assay provides a better method to assess indoor fungal exposure.

In contrast to occupant-reported mold and/or damp spots, no relations between investigator-observed damp and/or mold spots and EPS-Asp/Pen concentrations were found. We previously showed a similar difference when the relationship between observer- and occupant-reported dampness and Der p I and culturable fungi was studied.^{11,23} Apparently, occupant reports are more reliable estimates for dampness than observer reports.

The present study suggests a positive association between EPS-Asp/Pen levels in living room floor dust and respiratory symptoms and a reversed association with EPS-Asp/Pen levels in bedroom floor and mattress dust. This reversed association may also be due to allergen-avoidance measures. We previously showed that in the larger population, from which the present subpopulation was recruited,²⁴ allergen avoidance measures biased the relation between Der p I concentrations and respiratory health parameters. In that study, ORs increased and *P* values decreased substantially after subjects for whom allergen avoidance measures had been taken in their homes were excluded. This was particularly the case for relations with Der p I levels in bedroom floor and mattress dust. Allergen avoidance measures were mainly taken in the bedroom, because carpeted bed-

room floors and mattresses are generally assumed to contribute most to mite allergen exposure. Floor covers in living rooms were much less frequently changed. A similar pattern was also noted in this study. Allergen avoidance measures as an explanation for the reversed associations could, however, not be confirmed in our study. A restriction of the analyses to bedrooms where no allergen avoidance measures had been taken resulted in only marginally increased ORs. Larger, and preferably prospective, studies are required to allow proper adjustment for allergen-avoidance measures and further elucidate the relationship between EPS-Asp/Pen and respiratory symptoms.

In this study EPS-Asp/Pen levels in living room floor dust were associated with reported signs of mold growth and home dampness and with respiratory symptoms. In spite of the interpretation problems, our study thus seems to confirm various other epidemiologic studies that have consistently demonstrated an association between reported mold growth and home dampness and respiratory symptoms.^{2-5,32-34} Of these studies, only a few, however, have used actual exposure measurements.^{12,29,30}

We conclude that the EIA for fungal EPS-Asp/Pen may be a useful method for indoor fungal exposure assessment, with adequate sensitivity and reproducibility. The new assay may thus provide a useful epidemiologic tool to further investigate the relationship between damp homes, fungal exposure, and respiratory health.

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