

Fungal propagules in house dust. I.

Comparison of analytic methods and their value as estimators of potential exposure

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The presence of viable mold propagules in house dust was investigated by 10 different analytic methods, in order to determine to what extent different results are obtained when different analytic methods are used. Moreover, the value of this measurement as an estimator of the potential exposure to fungi in epidemiologic studies was assessed. Floor and mattress dust was sampled in 60 homes in The Netherlands during autumn 1990. For investigation of the variability in time, sampling was repeated in 20 homes after 6 weeks. Each analytic method is characterized by a unique combination of culture medium, suspension medium, and dilution step. The highest mean number of colony-forming units (CFU)/g dust was obtained by suspension of at least 100 mg dust in a peptone or sucrose solution in a ratio of 1:50 (w/w), followed by 10-fold dilution and plating on DG18 agar (geometric mean (GM) approximately 60 000 CFU/g dust). The lowest mean number of CFU/g dust was obtained by direct plating of 30 mg dust on V8 agar (GM approximately 5300 CFU/g dust). The mean coefficient of variation of duplicate analyses varied from 11%, for suspension in sucrose and plating on DG18 agar, to 27%, for suspension and dilution in sucrose in combination with V8 agar. The highest mean number of species isolated was obtained by direct plating of 30 mg dust on DG18 agar (mean number of species: 17). Suspension and dilution on DG18 or V8 agars yielded an average of approximately six species. In duplicate analyses, the mean percentage of agreement for the species isolated varied from approximately 35%, for suspension and dilution, to 60%, for direct plating. The reproducibility of the number of CFU/g dust in time was better for mattress dust than for floor dust; however, also for mattress dust, the predictive value of a single measurement was rather low. The variability in time in species isolated was substantial, both for mattress dust and floor dust. We concluded that results of measurements of viable mold propagules in house dust, both quantitatively and qualitatively, depend greatly on the analytic methods used. Furthermore, a single measurement of fungal propagules in settled house dust does not provide a reliable measure of potential exposure to fungi in indoor environments.

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Fungi and house-dust mites are thought to be a major cause of respiratory symptoms in damp houses. The ability to assess human exposure to fungi and house-dust mites might clarify the relation between living in damp houses and possible health effects, as reported in a number of recent epidemiologic studies (2, 4, 7, 8, 18, 20, 26, 27, 30). The major allergens produced by house-dust mites are well characterized and standardized (21, 22). There is therefore the possibility of assessing human exposure to these allergens indirectly by measuring the allergen content in settled

house dust. Isolation, purification, and standardization of the major fungal allergens are still beset by major problems (5, 16). Furthermore, the available methods for the measurement of fungal propagules (viable fungal particles) in indoor air do not provide accurate data for an estimation of the exposure to fungi in indoor environments. This is due to the large variability in time, even within very short time periods (13, 28). Overall, the variation of airborne fungal propagules in time is larger within homes than between homes (29). Therefore, air sampling of

viable fungal particles does not provide a reliable assessment of the long-term exposure of occupants to fungi in indoor environments.

Like the measurement of house-dust mite allergens in settled house dust, the measurement of fungal propagules in settled house dust might also give a useful measure of potential exposure to fungi. Most reports on the presence of mold propagules in house dust are based on various analytic methods. In the studies of Gravesen (10), Wood et al. (33), and Wickman et al. (32), 30 mg dust was plated directly onto a medium. In other studies (14, 17, 19, 24, 25, 31, 34), the dust was suspended in a solution and diluted stepwise before being plated onto a medium. Furthermore, different culture media were used. The results of measurements of airborne fungal propagules depend greatly on the air-sampling device and culture medium used (e.g., 6, 11, 28). The question arises of whether the results of different analytic methods of measuring the presence of fungal propagules in settled house dust also depend on the analytic method used. The present study was undertaken to document the extent to which results differ with the use of different analytic methods, to investigate the variability within and between homes of the presence of fungal propagules in house dust, and to assess the reliability of this method as a measure of potential exposure.

Material and methods

House-dust samples

House dust was sampled in 60 homes in The Netherlands. The homes were selected from a group of approximately 250 homes, included in a case-control study on home dampness and respiratory symptoms of children.

Dust was obtained from the bedroom floor and the mattress of the child with a vacuum cleaner (Philips Topomatic T518) equipped with a special attachment (the ALK allergen mouthpiece, ALK, Horsholm, Denmark) to collect dust on a cellulose filter (Schleicher & Schuell, 589¹ black ribbon 70 mm). The power of the vacuum cleaner was set at 1000 W. From each bedroom floor, an area of 2 m² was sampled for 4 min. The entire upper mattress surface with an area of approximately 2 m² was vacuum-cleaned for 2 min after the bedding had been removed. Between the samplings, the mouthpiece was thoroughly cleaned with 70% ethanol.

After sampling, the filters were placed in plastic Petri dishes and stored with silicagel in the dark at room temperature (for no more than 2 weeks) until analysis. Sampling was performed in October–November 1990. For investigation of the variability in time, sampling was repeated after a period of 6 weeks in the first 20 houses.

Analysis of the samples for fungal propagules

The various methods used to investigate the presence of fungal propagules in house dust are described below. Each method is a unique combination of culture medium, suspension fluid, and dilution step.

Direct plating. A 30-mg representative aliquot of unsieved dust was plated directly on either DG18 agar (12) or V8 agar (10), amended with chloramphenicol (100 mg/l) to prevent bacterial growth, with a sterile glass spreader, needle, and forceps. The water activity (a_w) of DG18 agar was 0.955 and of V8 agar 0.988.

Suspension in sucrose. At least 100 mg of representative dust was suspended in a 1:50 (w/w) sucrose solution (50% sucrose + 0.02% Tween 80 in distilled water, sterilized for 30 min at 110°C), shaken at 150 rpm for 10 min, and plated (0.1 ml) onto DG18 and V8 agars. This primary solution was diluted by a factor of 10 and, after shaking, 0.1 ml of this dilution was plated onto both agars.

Suspension in peptone. At least 100 mg of representative dust was suspended in a 1:50 (w/w) peptone solution (0.1% peptone (Difco) + 0.85% NaCl + 0.02% Tween 80 in distilled water). After shaking as above, 0.1-ml aliquots of the primary suspension and of a 10-fold dilution were plated onto DG18 and V8 agars.

As far as possible, all analyses were done in duplicate, for which each dust sample had to contain at least 320 mg dust. When less dust was gathered, only direct plating was done, and, if possible, suspension in sucrose. All methods described were applied to the samples taken in the first 20 homes. The samples obtained in the remaining 40 homes were analyzed only by direct plating on DG18 and V8 agars and by suspension in sucrose in combination with DG18 agar.

The plates were incubated in the dark for 5 days at 25°C. The number of colony-forming units (CFU) was counted by the naked eye with a colony counter (Gallenkamp, Loughborough, UK) and expressed as CFU/g dust, followed by transfer of inoculum from the colonies onto appropriate media for identification to species level according to standard mycologic procedures.

Statistical analyses

Statistical analysis of the data was done with SPSS-PC version 4.01. Pearson correlation coefficients were calculated to assess the association between the results in CFU/g dust obtained with the

various analytic methods. Paired *t*-tests were done to test differences between means in CFU/g dust and in number of species isolated. The reproducibility in time of the number of CFU/g dust was assessed by calculating the reliability coefficients from the two-way random effects ANOVA model, according to Armstrong et al. (1).

Results

Results in CFU/g dust

The numbers of CFU/g dust obtained with the various analytic methods varied widely. The distributions were right-skewed and became normally distributed after ln-transformation. Table 1 presents the number of CFU/g dust (geometric mean, geometric standard deviation, and range) obtained with the various analytic methods. Approximately 10% of the duplicate samples obtained by direct plating onto V8 and DG18 agars could not be counted because of the high density of the colonies on the agar plates. Furthermore, some dust samples did not contain sufficient dust for all the various analyses.

Statistically significant Pearson correlation coefficients were found between the numbers of CFU/g dust obtained with the various analytic methods. The correlation coefficients ranged from 0.39 ($P < 0.01$) between direct plating onto DG18 and suspension in peptone in combination with V8 agar, to 0.86 ($P < 0.001$) between suspension in peptone in combination with DG18 and suspension and dilution in peptone in combination with DG18 agar.

The highest numbers of CFU/g dust (Table 1) were obtained by suspension in sucrose or peptone, followed by a 10-fold dilution, in combination with DG18 agar. The lowest number of CFU/g dust was obtained by direct plating onto V8 agar. The yields obtained by direct plating onto both media were significantly lower (paired *t*-test, $P < 0.001$) than those obtained after suspension and/or dilution in sucrose or peptone. The yields after primary suspen-

sion were significantly lower ($P < 0.001$) than those after suspension and dilution. Comparisons were also made between the numbers of CFU/g dust obtained with different agars and different suspension fluids. The yields in CFU/g dust on DG18 agar were significantly higher than the corresponding yields on V8 agar, for both direct plating and plating of suspensions. The composition of the suspension fluid did not significantly influence the number of CFU/g dust.

The coefficients of variation of the duplicate analyses for the 10 analytic methods were calculated with the nontransformed data (Table 1). The mean coefficients of variation varied from 11.4% (primary suspension in sucrose in combination with DG18) to 27.2% (suspension and dilution in sucrose in combination with V8). Overall, the variation coefficients were higher after suspension and dilution than those after direct plating and primary suspension.

Species isolated

Because of the high density of the colonies on the agar plates or overgrowth by the fast-growing genera *Mucor* and *Rhizopus*, the colonies from approximately 30% of plates obtained by direct plating could not be isolated. With the other methods, this occurred in 0–10% of the samples. Overgrowth by *Mucor* and *Rhizopus* occurred mainly on V8 agar.

In total, 108 different species belonging to 54 different mold genera were isolated. The most frequently isolated species from mattress dust were *Alternaria alternata*, *Aspergillus versicolor*, *Aureobasidium pullulans*, *Botrytis cinerea*, *Cladosporium cladosporioides*, *C. herbarum*, *Epicoccum nigrum*, *Fusarium* spp., *Mucor* spp., *Penicillium brevicompactum*, *P. chrysogenum*, *P. glabrum*, *Phoma* spp., *Scopulariopsis brevicaulis*, *Ulocladium chartarum*, and some yeasts. From floor dust, the same mold species, except for *S. brevicaulis*, were isolated most frequently, as well as *C. sphaerospermum*, *Eurotium herbariorum*,

Table 1. Number of CFU/g dust (geometric mean (GM), geometric standard deviation (GDS), range) obtained with 10 different analytic methods, and corresponding coefficients of variation (mean, standard deviation)

Analytic method	Number of CFU/g dust				Coefficient of variation (%)		
	<i>n</i>	GM	GSD	Range	<i>n</i>	Mean	SD
V8 direct	140	5310	1.96	730–41440	116	12.5	11.8
DG18 direct	146	7470	2.14	980–56050	121	14.2	12.3
V8 peptone	52	28830	2.09	6000–130480	51	15.3	14.0
V8 sucrose	58	28770	2.22	5740–248950	57	16.4	14.9
DG18 peptone	52	36900	2.25	6750–140510	52	12.0	11.2
DG18 sucrose	109	36980	2.71	2120–360770	107	11.4	10.0
V8 peptone (10^{-1})	52	46680	2.82	2500–407580	51	26.2	21.8
V8 sucrose (10^{-1})	59	47810	3.16	6370–475920	59	27.2	23.2
DG18 peptone (10^{-1})	52	60170	2.76	10000–632230	51	24.3	20.3
DG18 sucrose (10^{-1})	109	59460	3.43	2130–1102400	109	24.9	22.8

Table 2. Number of species isolated (mean, standard deviation) for the different analytic methods and the reproducibility in terms of species isolated from duplicate analysis, on basis of samples from which all colonies were isolated and identified by species

Method	n	Number of different species		Agreement rate (%)	
		Mean	SD	Mean	SD
V8 direct	74	14.9	3.6	59.7	13.9
DG18 direct	88	17.3	4.2	61.0	11.9
V8 peptone	49	11.4	3.1	50.7	17.6
V8 sucrose	51	13.0	4.7	57.4	18.2
DG18 peptone	51	13.4	4.1	53.8	16.4
DG18 sucrose	106	13.2	4.4	54.5	17.4
V8 peptone (10 ⁻¹)	50	4.8	2.5	39.4	38.9
V8 sucrose (10 ⁻¹)	58	6.0	3.4	35.4	35.9
DG18 peptone (10 ⁻¹)	50	5.7	3.3	36.6	22.8
DG18 sucrose (10 ⁻¹)	107	7.0	3.8	35.5	20.2

Rhizopus spp., and *Wallemia sebi*. The following yeasts were predominant: *Rhodotorula glutinis*, *R. minuta*, *R. mucilaginosa*, *Cryptococcus albidus*, and *C. laurentii*.

The highest mean number of species isolated was found after direct plating onto DG18 agar (Table 2). The numbers of species obtained after suspension and dilution were significantly lower ($P < 0.001$, paired *t*-test) than those obtained by suspension only. The numbers of species obtained after suspension were significantly lower than those obtained by direct plating. Comparisons were also made between the numbers of species isolated with different agars and suspension fluids. The numbers of species isolated with DG18 agar were significantly higher than those obtained with V8 agar. Furthermore, suspension in sucrose yielded significantly more species than suspension in peptone. Xerophilic fungi, i.e., *Eurotium* spp. and *W. sebi*, were isolated more frequently on DG18 agar, whereas *Fusarium* spp. and *Scopulariopsis* spp. were isolated more frequently on V8 agar.

The reproducibility of the duplicate analyses in terms of species isolated was calculated as the agreement rate: the ratio of the number of identical species isolated from both plates and the total number of species isolated from both plates (Table 2). The mean agreement rate varied from approximately 37%, for suspension and dilution, to approximately 60%, for direct plating.

Variability in time

For information about the variability in time of the presence of fungal propagules in house dust, sampling was repeated in 20 homes after 6 weeks. For mattress dust, there were no statistically significant differences (paired *t*-test) between the average numbers of CFU/g dust found in both periods by most analytic methods, except for direct plating onto V8 agar and suspension in sucrose in combination with

both agars. For floor dust, the mean numbers of CFU/g dust of the first sampling period were significantly higher than those of the second sampling period, except for suspension in peptone in combination with V8 agar and suspension in sucrose in combination with both agars.

The reproducibility of the number of CFU/g dust in time was assessed by calculating the reliability coefficient from the two-way random effects ANOVA model (1). For the number of CFU/g in mattress dust, the reliability coefficients ranged from 0.06 to 0.74 (Table 3). For floor dust, the reliability coefficients ranged from 0.01 to 0.32. Several reliability coefficients were calculated as negative and are therefore not presented.

The agreement rate over time was calculated between the species isolated from samples taken during the first and second sampling periods. Only samples in which all colonies were identified at species level were included. The results of duplicate analyses per sampling period were paired randomly. The mean agreement rates for mattress dust and floor dust are presented in Table 4. For mattress dust, the highest mean agreement rates (approximately 43%) between the species isolated in both

Table 3. Estimated reliability coefficients for number of CFU/g dust, for mattress dust and floor dust separately

Method	Mattress	Floor
V8 direct	0.52	0.32
DG18 direct	0.19	0.21
V8 peptone	0.06	0.20
V8 sucrose	0.74	*
DG18 peptone	*	*
DG18 sucrose	0.51	*
V8 peptone (10 ⁻¹)	*	0.01
V8 sucrose (10 ⁻¹)	0.58	*
DG18 peptone (10 ⁻¹)	*	0.05
DG18 sucrose (10 ⁻¹)	0.42	*

* Negative reliability coefficient, due to larger within-home mean square of variance than between-home mean square of variance.

Table 4. Agreement rate over time (%) between species isolated from samples taken in 20 homes, within period of 6 weeks, on basis of plates from which all colonies were identified

Method	Agreement rate (%)					
	Mattress			Floor		
	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD
V8 direct	6	42.4	11.4	2	29.2	20.9
DG18 direct	10	44.0	8.2	4	40.5	6.6
V8 peptone	24	34.9	14.9	21	39.9	10.1
V8 sucrose	22	35.2	12.1	19	43.0	10.7
DG18 peptone	24	34.9	13.0	24	38.7	9.4
DG18 sucrose	24	33.4	11.6	26	41.8	10.1
V8 peptone (10 ⁻¹)	24	17.0	16.3	23	23.1	18.8
V8 sucrose (10 ⁻¹)	24	18.6	14.8	29	27.5	15.9
DG18 peptone (10 ⁻¹)	24	14.8	20.2	24	22.3	15.4
DG18 sucrose (10 ⁻¹)	24	21.7	13.1	29	27.5	10.1

sampling periods were found for direct plating. However, the number of samples was limited. For floor dust, the highest mean agreement rates (approximately 40%) were obtained for suspension, irrespective of the composition of the suspension fluid or culture medium.

Discussion

The comparison between the yields of CFU/g dust indicated that the highest numbers were obtained by suspension followed by 10-fold dilution, and the lowest by direct plating. This was most probably due to breaking up of aggregates of spores during suspension and dilution, which also occurs with the dilution plate count method for the enumeration of fungi in soil (15). Furthermore, the factors used to calculate the numbers of CFU/g dust vary widely for the various analytic methods, e.g., from 33.3 for direct plating, to 5000, for suspension and dilution in peptone. In studies using suspension and dilution to assess the presence of fungal propagules in house dust, (very) high numbers of CFU/g dust up to 7×10^6 have been reported (24). In studies where the dust was analyzed by direct plating of 30 mg dust (32, 33), the median values were around $1-2 \times 10^3$.

The composition of the suspension solution (peptone or sucrose) did not influence the yield of CFU. The agar did have an influence; the yield on DG18 was significantly higher than on V8 agar. However, the results obtained with the various analytic methods were highly correlated. van de Lustgraaf (17) compared the yields for the isolation of xerophilic fungi in mattress dust. He used three different agars: malt extract agar with 8% NaCl, 40% sucrose, or 64% sucrose (a_w , respectively, 0.94, 0.94 and 0.82). Dust samples were suspended in a 1:50 (w/w) sucrose solution (50% sucrose plus 0.02% Tween 80), and aliquots of 0.1 ml were plated onto the agar plates. Overall, the lowest counts were obtained on

malt extract agar with 64% sucrose (mean approximately 7000 CFU/g dust). The highest counts were found on malt extract agar with 40% sucrose (mean approximately 20 000 CFU/g dust). Miller et al. (19) reported higher counts on rose bengal 2% malt extract agar (mean 8×10^5 CFU/g dust) than malt extract agar with 20% sucrose (mean 5×10^5 CFU/g dust). Dust was suspended in sterile water and diluted by a factor of 10^6 . A comparison between the yields in CFU/m³ with different air-sampling devices and agars, including DG18, also showed higher numbers on DG18 (28). To our knowledge, no other comparative studies of the yields using DG18 and V8 agar have been published.

For direct plating, identification of the colonies was impossible for approximately 30% of the plates, especially on V8 agar, because of the high density of colonies on the plates or overgrowth by fast-growing genera. When information on fungal species in house dust is required, direct plating might not be appropriate. Nevertheless, when it was possible to isolate the colonies, the highest mean number of species was obtained by direct plating. The number of species isolated on DG18 was significantly higher than on V8 agar. This confirms the value of DG18 reported in an earlier comparative study of species in indoor air, in which higher yields were also obtained when DG18 was used as isolation medium (28). Xerophilic fungi, i.e., *Eurotium* spp. and *Wallemia sebi*, were isolated more frequently on DG18 agar. In the study of van de Lustgraaf (17), xerophilic fungi were isolated most frequently on malt extract agar with 64% sucrose. However, with this medium, other common species in house dust, such as *Cladosporium* spp. and *Aureobasidium* spp., were not found. Besides the medium, the composition of the suspension solution also had a significant influence on the yield of species isolated. Suspension in sucrose yielded higher numbers of species than suspension in peptone.

We conclude that the results of the measurements of fungal propagules in house dust, both quantitatively and qualitatively, depend greatly on the analytic method used.

The reproducibility in terms of CFU/g dust of duplicate analyses was acceptable for direct plating and the primary suspension, with mean coefficients of variation of approximately 11–16%. For the species isolated, the reproducibility of duplicate analyses was only moderate: the highest mean agreement rate found was approximately 60% (direct plating).

Overall, the reproducibility of the number of CFU/g dust in time was better for mattress dust than floor dust; however, even for mattress dust, the predictive value of a single measurement was low. This difference might be explained by varying cleaning methods. Mattress dust is probably less frequently disturbed by cleaning than floor dust. The variability with time in the species isolated is substantial, for both mattress dust and floor dust. Thus, a single sample of house dust does not provide reliable information on the presence of fungal propagules (quantitatively as well as qualitatively) over a longer period. Therefore, the potential exposure can be assessed only to a limited extent. Brunekreef et al. (3) pointed out that the error in the exposure assessment in epidemiologic studies on the relationship between environmental pollution and health effects reduces the power of a study to detect a significant association and might introduce a bias in estimates of the size of an effect. Our results do not confirm the suggestion made by Miller et al. (19) that a single sample of house dust reflects the presence of fungal propagules (quantitatively) over a long period. They found that the number of CFU/g dust in dust sampled in a new vacuum bag differed by only 10% from that found in a sample taken from the vacuum bag present in the vacuum cleaner at the moment of sampling. Miller et al. (19) did not provide information on the presence of fungal species.

We conclude that a single sample of settled house dust for the assessment of fungal propagules does not provide a reliable measure of the potential exposure to fungi in indoor environments. Earlier we concluded that measurements of airborne fungal propagules in nonindustrial indoor environments show the same lack of reliability for the estimation of exposure to fungi (29). Therefore, other indicators of exposure to fungi in indoor environments should be considered. For example, sampling of cell-wall components of fungi, such as 1,3- β -glucan and lipopolysaccharides, might give an overall estimate of airborne fungal matter. Glucan can be assayed by a modification of the procedure used for bacterial endotoxin (23). Further evaluation of its value as a measure of exposure to fungi is necessary. However, a disadvantage of using glucans as an indicator of

exposure to fungi is that no information on the fungal species present can be obtained. Fungi also produce volatile compounds, frequently evident as a "moldy smell". The principal volatile produced by various fungi is 1-octen-3-ol (9). Miller et al. (19) suggested that 3-methyl-1-butanol, 2-hexanone, and 2-heptanone are possibly more indicative of fungal activity. However, the measurement of these volatiles as a measure of exposure to fungi has to be further evaluated, because these compounds may have other indoor sources as well.

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