

Letter to the Editor

Objective assessment of domestic mold contamination using quantitative PCR

To the Editor:

Exposure to indoor fungi has the potential to exacerbate and contribute to the onset of asthma.^{1,2} Visible mold assessments by building occupants are used extensively in large research studies; however, such self-reports can be subjective and mold can be missed. Using independent building inspectors can make mold assessments more objective, but it can be time consuming and expensive. Exposure assessment of fungi using microbial or molecular determination from indoor samples is complicated by measurement issues.³ Collected house dust, such as vacuumed floor dust or mattress dust, is a convenient sample commonly used in epidemiological studies,³ but the representativeness of such samples for inhalation exposure has been challenged.^{4,5} Traditional active air sampling for airborne fungi requires special equipment, is costly and hard to implement in larger epidemiological studies, and typically relies on sampling, which due to its short-term nature shows limited repeatability.⁶ Measures of viable spores are also problematic because nonviable spores and cell fragments may still contribute to ill health. Thus, no valid criterion standard exists for indoor mold exposure assessment. We trialed a novel system similar to that previously described by Noss et al.⁴ for endotoxin assessment, to measure indoor airborne levels of fungi and bacteria with quantitative PCR (qPCR). We used an electrostatic dust cloth to passively collect airborne microbes over a period of 4 weeks, and compared this assessment method to traditional assessment methods of visible mold.

Electrostatic dust cloths (Pledge Grab-It Dust & Allergen 29.8 cm × 15 cm; SC Johnson, Racine, Wis) were placed in the bedrooms, using a shelf attached to the bedroom wall (1.5 m height), of 300 children who were enrolled in an incident asthma case-control study in New Zealand. The cloths were left in place for 4 weeks. Ethical approval was obtained from the Central Health and Disability Ethics Committee (CEN-09-06-039). Assessment of visible mold in the children's bedrooms was carried out by researchers trained in mycological identification. Visible mold was assessed in 7 locations in the child's bedroom (walls, ceilings, floors, windows, curtains, bedding, and wardrobes), and the extent of visible mold was also rated on a mold severity scale using a show card, ranging from 0 to 3 (0, no visible mold; 1, minor visible mold <10 cm length; 2, moderate visible mold between 10 and 50 cm; 3, major visible mold >50 cm). A mold score (0-21) was calculated by summing the extent scores for each location of mold. Homes with a mold score of 0 were categorized as having "no mold," those with a score of 1 to 3 as having "medium" levels of visible mold, and those with a score of 4 to 21 as having "high" levels of visible mold. Researchers and parents also reported on other dampness factors including the presence of water damage/leaks, mold odor, and condensation in the child's bedroom. Electrostatic dust cloths (n = 299; 1 cloth defrosted and not analyzed) were stored frozen and remained frozen until analysis, including during transportation to the National Institute for Health and Welfare, Finland. Samples were extracted 2 consecutive times in 50 mL sterile water with 0.05% Tween20. The resulting extracts were concentrated using centrifugation and subjected to bead milling for mechanical cell disruption and subsequent DNA purification as described

previously⁷ with minor modifications, using a KingFisher DNA extraction robot (Thermo Fisher Scientific, Vantaa, Finland), NucleoMag plant kit (Macherey Nagel, Dueren, Germany), and salmon testis DNA as an internal control. Using qPCR developed and described previously,^{7,8} the total fungal DNA (targeting most species within the fungal kingdom), as well as DNA of *Penicillium* species/*Aspergillus* species/*Paecilomyces variotii* group, *Cladosporium cladosporioides*, and *Aspergillus versicolor*, and Gram-positive and Gram-negative bacteria levels were assessed. Determination of Gram-negative bacteria was hampered by background DNA contamination in the magnetic beads of the NucleoMag plant kit; such issues can be avoided by using DNA purification kits with specially treated beads (eg, Chemagic DNA plant kit with Dnaex-treated beads; PerkinElmer, Baesweiler, Germany). Statistical analysis in R (version 2.15.2) used the log-transformed microbial measurement data to compare with mold score by using Pearson correlation test, and for moisture damage measures of presence/absence by using Wilcoxon signed ranked tests.

Fungal and bacterial levels found by using qPCR in static dust cloths varied in magnitude in a dose-dependent way with researcher-assessed levels of none, medium, and high visible mold in children's bedrooms (Table I; $P \leq .05$ for trend). These findings were significant for qPCR markers of total fungi and *Penicillium* species/*Aspergillus* species/*P. variotii* group ($P \leq .05$). Microbial levels of fungi and bacteria found by using qPCR were also significantly associated with individual researcher and parental observations of dampness and moisture damage, with Pearson r values ranging between 0.14 and 0.40 (Table II; $P \leq .05$). Of the microbial markers investigated, total fungi and *Penicillium* species/*Aspergillus* species/*P. variotii* levels were significantly associated with researcher and parental observations of mold and dampness markers, including visible mold score, mold odor, visible dampness by parents, and leaks and moisture damage by researchers (Table II; $P \leq .05$). *C. cladosporioides* levels were also associated with parental and researcher observations of dampness and mold, but associations were weaker, likely due to the strong outdoor context of *Cladosporium* species in general. *Aspergillus versicolor*, a fungal species often considered a dampness indicator, was detected in only 7% of the cloths, but was significantly more prevalent in "high mold" homes ($P \leq .05$). Gram-positive bacteria were significantly associated with researcher observations of leaks and moisture damage ($P \leq .05$). In addition to visible mold score, parental assessments of mold odor, which have been found in other studies to be related to potential health effects,^{2,9} were positively associated with all fungal qPCR groups.

Use of qPCR analyses from electrostatic dust cloth sampling for settled dust is a promising tool to objectively measure microbial contamination in residential homes, supporting earlier findings on the use of static dust cloth collectors.^{4,5} Because the cloth method can be applied for extended periods of time, they overcome a major disadvantage of short-term active air sampling, which is highly sensitive to large temporal fluctuations in airborne microbial levels. The cloths require no mycological training to set up and can be applied by the study subjects themselves in their homes. This sampling approach is suitable for large-scale epidemiological studies as an objective measure of

TABLE I. Median levels (expressed as cell equivalents per cloth, minimum and maximum values in parentheses) of fungi and bacteria found by using qPCR in static dust cloths categorized by researcher-assessed low, medium, and high levels of visible mold in the child's bedroom

Visible mold score category	Total fungi (n = 299)*	<i>Penicillium/Aspergillus</i> group (n = 299)*	<i>Cladosporium cladosporioides</i> (n = 299)*	Gram-positive bacteria (n = 299)*
No visible mold (n = 108)	35,610 (2,160-312,100)	18,650 (632-349,500)	256 (12-12,780)	1,631,000 (13,980-47,030,000)
Medium visible mold score (n = 142)	54,840 (1,740-748,900)	36,420 (858-912,800)	301 (19-14,560)	2,496,000 (26,040-44,920,000)
High visible mold score (n = 49)	77,150 (916-1,192,000)	68,220 (822-1,392,000)	580 (11-9,808)	2,806,000 (7,000-34,920,000)

* $P \leq .05$ for trend.**TABLE II.** Pearson correlation values and significant positive associations of levels of fungi and bacteria found by using qPCR in static dust cloths and observations of visible mold and dampness by trained researchers and parents

Mold/ moisture damage category	Total fungi (n = 299)	<i>Penicillium/Aspergillus</i> group (n = 299)	<i>Cladosporium cladosporioides</i> (n = 299)	Gram-positive bacteria (n = 299)
Visible mold score				
Researcher	0.28*	0.40*	0.14†	0.07
Parent	0.16*	0.25*	0.09	0.04
Mold odor				
Researcher	0.18*	0.27*	0.11†	0.06
Parent	0.20*	0.25*	0.19‡	0.09
Leaks/moisture damage				
Researcher	0.20*	0.25*	0.08	0.14†
Parent	0.11	0.05	0.16‡	0.03
Signs of condensation				
Researcher	0.03	0.09	0.02	0.02
Parent	0.00	0.06	-0.03	-0.03
Visible dampness				
Parent	0.16‡	0.23*	0.06	0.06

* $P \leq .001$.† $P \leq .05$.‡ $P \leq .01$.

microbial exposure, and to supplement exposure information gathered by building inspections for dampness and mold. However, further analysis is needed to examine whether the levels of fungi and bacteria found by using qPCR are related to health measures and is a more valid exposure assessment tool than subjective self-reported exposures.

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