

# Occurrence of Household Mold and Efficacy of Sodium Hypochlorite Disinfectant

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*The occurrence and distribution of mold on household surfaces and the efficacy of bleach-based (sodium hypochlorite, NaOCl) disinfectants on mold viability and allergenicity was documented. Household microenvironments prone to increased moisture were specifically targeted. Using the sticky tape method, 1330 samples were collected from non-porous indoor surfaces of 160 homes across the United States, and analyzed for mold. Homes were randomly selected and recruited via phone interviews. Culture and immunoassays were used to measure the viability and reduction of allergenic properties of *Aspergillus fumigatus* following 2.4% NaOCl treatment. All homes and 72.9% of surfaces tested positive for mold. Windowsills were the most frequently contaminated site (87.5%) and *Cladosporium* the most commonly identified mold (31.0%). Five-minute exposures to 2.4% NaOCl resulted in a >3 to >6-log<sub>10</sub> reduction of culturable mold counts in controlled laboratory studies. Organisms were nonculturable after 5- and 10-min contact times on non-porous and porous ceramic carriers, respectively, and *A. fumigatus* spore-eluted allergen levels were reduced by an average 95.8% in 30 sec, as indicated by immunoassay. All homes are contaminated with some level of mold, and regrowth is likely in moisture-prone microenvironments. The use of low concentrations (2.4%) of NaOCl for the reduction of culturable indoor mold and related allergens is effective and recommended.*

**Keywords** allergens, disinfectant, fungi, household hygiene

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## INTRODUCTION

Mold is commonly found indoors and is associated with a wide range of health effects in humans.<sup>(1–5)</sup> Most of the literature focuses on mold remediation strategies following severe flooding events,<sup>(6,7)</sup> with little information available regarding mold occurrence and control in the absence of severe events. Mold exposures and human health risks are

not well characterized, and threshold values are not quantified.<sup>(8,9)</sup> However, occupants of buildings and homes prone to excessive moisture are at higher risk for developing mold-related allergies, asthma, and other respiratory disorders.<sup>(10–15)</sup> Furthermore, approximately 10% of the population expresses high levels of antibodies to common indoor molds,<sup>(16)</sup> with 5% predicted to experience symptoms due to mold exposures.<sup>(17)</sup> According to a nationally representative sample of children from the Third National Health and Nutrition Examination Survey (NHANES III),<sup>(18)</sup> 16% of children aged 6–16 were sensitized to *Alternaria alternata*. Previous studies report that 27 to 36% of homes have mold or moisture problems, while indoor air quality measurements estimate a higher prevalence of up to 56%.<sup>(5,10,19,20)</sup>

Although several studies document the efficacy of bleach-based products for the reduction of culturable mold, mold allergens, and other household allergens,<sup>(21–25)</sup> recommendations for biocide use to control indoor mold are generally lacking from state and federal public health guidelines.<sup>(26,27)</sup> Others continue to report the use of bleach to abate mold as controversial.<sup>(28)</sup> To date, little information has been published relative to the occurrence of mold on various indoor home surfaces and the efficacy of household cleaning products for the reduction of mold and mold allergens from the home environment. Therefore, the objectives of this study were to: (1) document the relative occurrence and distribution of potentially harmful mold on indoor home surfaces, and (2) use environmental mold isolates from Objective 1 and commercially available mold stocks to determine the efficacy of a 2.4% NaOCl disinfectant on viability and allergenicity.

## METHODS

### Home Mold Survey

To survey for mold contamination on indoor household surfaces, 1330 samples were collected from June to August in homes from five regions, represented by seven cities in

the United States including: the West (San Francisco, California), Southwest (Tucson, Arizona; Dallas, Texas), Midwest (Chicago, Illinois), Southeast (Atlanta, Georgia; Tampa, Florida), and Northeast (New York City). Household residents over the age of 18 ( $n = 160$ ) were randomly solicited via phone interviews by a professional recruiting agency. Targeted surfaces included air vents, windowsills, entryway floors, under kitchen sinks, refrigerator seals, shower grout, and shower ceilings. Residents sampled surfaces using contact slide kits comprising 10 plastic microscope slides, 10 clear sticky tape-lift samplers, and an instruction sheet. They were instructed to peel the tape off the paper backing, and without touching the tape, lightly contact the sticky side with the test area, lift away, then firmly press to the plastic microscope slide. Used slides were placed in individual envelopes labeled with the target site. One slide in each kit was used to test any environment the resident chose. Kits were returned to the laboratory within 5 days.

Slides were examined qualitatively using light microscopy at 100 $\times$  and 400 $\times$  magnification for the presence of *Alternaria*, *Aspergillus niger*, *Aspergillus* sp., *Penicillium*, *Chaetomium*, *Cladosporium*, *Helminthosporium*, *Rhizopus*, *Stachybotrys*, hyphal fragments, and other molds. Spores and hyphae were identified based on characteristic morphology. Due to difficulties in differentiating *Aspergillus* sp. and *Penicillium*, morphologically, results of these genera were combined.

## Disinfectant Efficacy

### Mold Stock Preparation

Disinfectant efficacy was evaluated according to the Association of Official Analytical Chemists International (AOAC) Germicidal Spray Products Disinfectant test protocol.<sup>(29)</sup> Non-porous (glazed) and porous (unglazed) ceramic carriers (2.5 cm<sup>2</sup>) were cleaned, sterilized, and assembled into individual paper-lined plastic petri dishes. Test organisms *Alternaria alternata* (American Type Culture Collection; ATCC 6663), *Aspergillus niger* (ATCC 16404), *Cladosporium herbarum* (ATCC 28987), *Mucor bainieri* (42642), *Penicillium chrysogenum* (ATCC 10106), *Stachybotrys chartarum* (ATCC 9182), and *Trichophyton mentagrophytes* (ATCC 26323) were cultured on recommended media<sup>(30)</sup> for 10 to 15 days at 26  $\pm$  2°C.

After incubation, 20 mL of Triton X-100 solution (0.05% Triton X-100, 0.85% sodium chloride) was added to each plate, and the fungal mycelia gently scraped with a sterile L-shaped spreader. The conidia-containing fluid was blended at high speed for 2 to 5 min in a commercial blender (Waring Products Division, New Hartford, Conn.), the suspension passed through sterile cheesecloth, and centrifuged at 2800  $\times$  g for 15 min (TJ-6 Beckman centrifuge; Beckman Coulter, Inc. Palo Alto, Calif.). The supernatant was removed and the pellet resuspended in 10 mL of Triton X-100 solution, centrifuged once more, and again resuspended in 1–2 mL of Triton X-100 solution. Harvested stocks were enumerated by direct hemacytometer count, and culture on potato dextrose-

yeast agar (PDYA, Sigma-Aldrich, St. Louis, Mo.). A working suspension of fungal conidia was prepared by diluting the stock to approximately  $1 \times 10^6$  conidia/mL in Triton X-100 solution containing 5% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, Mo.).

### Disinfectant Testing

Carriers were inoculated with 10 to 30  $\mu$ L of the organism suspension. On glazed ceramic carriers, the inoculum uniformly spread to within 3–5 mm of the edges. On unglazed ceramic carriers, the inoculum penetrated with minimal spreading. Contaminated carriers were air dried for >20 min until visibly dry.

Two batches of commercial bleach-based mold disinfectant (Tilex, Clorox Co., Pleasanton, Calif.) were purchased from a local grocery store with a concentration of 2.4% NaOCl. Ten individual carriers were sprayed to saturation using two to three pumps from a distance of 15 to 20 cm. Following 5-min contact on glazed and 10-min contact on unglazed ceramic carriers, each carrier was transferred to potato dextrose broth, with 0.5% yeast extract and 0.2% sodium thiosulfate (PDYB+). Bacterial growth was inhibited by addition of streptomycin (50  $\mu$ g/mL) to the media. After incubation at 26  $\pm$  2°C for 10 days, vessels were examined for turbidity and recorded as growth or no growth. Positive growth samples were examined microscopically to verify growth was due to seeded fungal colonies.

Mold isolates from home environments were grown from tape-lift samples using potato dextrose agar and malt extract agar. Colonies were isolated from culture plates after two successive passages and pure cultures grown to sporulation. Conidia were purified, enumerated, and dried onto 10 cm<sup>2</sup> non-porous porcelain tiles and treated with 2.4% NaOCl, as described above. The inoculum (up to  $10^7$  colony forming units [CFU]/tile) was allowed to dry for 20 to 30 min. Replicates of three to four tiles per mold were completed. Following a 5-min contact time, the area was sampled with a specimen swab saturated with neutralizer (0.85% NaCl, 0.1% Triton X-100 containing 0.3% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>). In experiments with both ATCC-purchased and environmentally isolated mold stocks, control samples utilizing untreated tiles (i.e., no disinfectant) were identically sampled and cultured to determine the effect of neutralizer on the mold counts, and a non-treated tile was sampled to enumerate the mold and establish the effect of drying on the original culture concentration. Culture plates were examined microscopically to verify culture purity.

### Fragmentation of Allergenic Proteins

Fragmentation analysis of allergenic proteins, following exposure to 2.4% NaOCl, was performed using two sources of *Aspergillus fumigatus* spore-eluted allergen. Two sets of spore-eluted allergen standard curves were produced using each source separately (*A. fumigatus*: ATCC 14110, and an *A. fumigatus* spore-based antigen control purchased from Gibson Laboratories, Lexington, Ky.).

### Standard Curve Preparation

Eight standards (initial 1:10 dilution of culture followed by six serial 1:2 dilutions in 2.4% NaOCl disinfectant neutralized with sodium sulfite and HCl, pH 7.4, and a disinfectant blank) were prepared. *A. fumigatus* spore concentrations were  $1.0 \times 10^7$ ,  $5.0 \times 10^6$ ,  $2.5 \times 10^6$ ,  $1.25 \times 10^6$ ,  $6.25 \times 10^5$ ,  $3.13 \times 10^5$ ,  $1.56 \times 10^5$ , and 0 spores present. *A. fumigatus* antigen allergen concentration was determined by subsequent cultural data.

### Test Sample Preparation

One mL of 2.4% NaOCl disinfectant was added to each spore-eluted ( $5 \times 10^6$  spores/50  $\mu$ L) allergen stock. The sample was vortexed and neutralized with 160  $\mu$ L of 2 M sodium sulfite after 30-sec, 1-min, or 5-min contact time. In addition, 78  $\mu$ L of 2 M HCl was added for a final pH of 7.4.

### Enzyme-Linked Immunosorbent Assay (ELISA)

Using a capture ELISA protocol, 96 well plates (Immulon 2 HB Plates, Dynex, Chantilly, Va.) were coated with goat anti-*A. fumigatus* (Immuno-Mycologics, Norman, Okla.). As per manufacturer's instructions, antibody was diluted 1:800, and 100  $\mu$ L added to each well. Following overnight incubation at 4°C, coated wells were washed two times with phosphate buffered saline with tween (PBS-T) and blocked by addition of 200  $\mu$ L of PBS-T with 1% bovine serum albumin, pH 7.4 (PBS-T/BSA), and washed two times with PBS-T. Antigen-containing standards and samples (100  $\mu$ L) were added to the plate, incubated for 1 hr at 37°C and washed three times with PBS-T. Rabbit anti-*A. fumigatus* (primary label) was diluted 1:800 in PBS-T and 100  $\mu$ L added to each well. Following 1-hr incubation at 37°C, wells were washed nine times with PBS-T. Anti-rabbit peroxidase conjugated immunoglobulin (secondary label) was diluted 1:5000 in PBS-T and 100  $\mu$ L added to each well followed by a 1-hr incubation at 37°C, and six PBS-T washes. For the detection of peroxidase activity, 100  $\mu$ L of *o*-phenylenediamine dihydrochloride was added to each well, and plates were incubated in the dark for 30 min at room temperature, followed by addition of 50  $\mu$ L of 3 M HCl stop reagent. The optical density of the sample well was determined using a 96-well plate reader and a single wavelength (490 nm) ELISA endpoint assay. Data were analyzed relative to a quadratic standard curve fit.

## RESULTS

### Home Mold Survey

One hundred percent (160/160) of the surveyed homes tested positive for mold. At least one type of mold was detected on 72.9% (970/1330) of all surface samples. Shower grout and shower ceilings were among the least frequently positive (49.0%; 75/153 and 50.0%; 58/116, respectively). Windowsills and refrigerator seals were the most frequently positive sites (87.5%; 126/144 and 83.3%; 105/126, respectively). Other sites were commonly contaminated, including air registers

(82.5%; 160/194), under the kitchen sink (82.9%; 102/123), and entryways (74.6%; 94/126).

Some of the sites tested positive for more than one type of mold; thus, 1092 mold isolates were identified on 970 positive sites. The most frequently identified mold was *Cladosporium* (31.0%; 339). Other molds, identified by decreasing prevalence, included *Alternaria* (14.4%; 157), *Helminthosporium* (9.0%; 98), *Aspergillus* sp. or *Penicillium* (2.8%; 31), and *Chaetomium* (1.6%; 17). *Stachybotrys* was found in 2/160 (1.2%) of the homes tested, equivalent to 0.2% of the identified mold. Both *Stachybotrys* positive samples were collected from entryways into the home, one in California and one in Arizona. No samples were positive for *A. niger*. Hyphal fragments were found in 33.2% (362) of the positive samples collected and constituted a major portion of the mold contaminants found on home surfaces. Approximately 52.6% (575) of positive samples contained other molds, i.e., those not on our target list of known health hazards.<sup>(31)</sup>

### Disinfectant Efficacy

The spores of all tested organisms from ATCC stock cultures were inactivated by 2.4% NaOCl to undetectable levels. Test organisms were nonculturable in 10/10 trials, after 5-min contact times in glazed ceramic carriers, and after 10 min in unglazed ceramic carriers, representing a  $>3\text{-log}_{10}$  to  $>5\text{-log}_{10}$  reduction, depending on the mold type and initial concentration of the dried inoculum (Table I). Control neutralization experiments showed that PDB+ effectively neutralized the antifungal activity of the test substance prior to culture analysis.

Similarly, environmental isolates of molds collected during the home survey were highly susceptible to 2.4% NaOCl (Table II). *Penicillium*, *Aspergillus*, and *Rhizopus* cultures grown from the tape-lift samples were exposed to 2.4% NaOCl for 5 min. The initial inoculum of  $10^7$ ,  $10^6$ , and  $10^6$ , respectively, was reduced to  $10^6$ ,  $10^5$ , and  $10^5$  in controls where a disinfectant was not used. Thus, an approximately  $1\text{-log}_{10}$  reduction was due to the sampling procedure, either to inefficient swabbing or loss of culturable spores due to drying. A 5-min exposure to the disinfectant resulted in a  $>5$  to  $>6\text{-log}_{10}$  reduction in mold CFU, following a 7-day incubation period at room temperature. Other environmental isolates (i.e., *Stachybotrys*, *Alternaria*, and *Helminthosporium*) could not be grown to titers above  $10^3$ . Approximately  $2\text{-log}_{10}$  CFU were lost due to the sampling procedure, resulting in  $>1$  to  $>2\text{-log}_{10}$  reductions following 2.4% NaOCl, or no detectable CFU.

### Fragmentation of Allergenic Proteins

Treatment of *A. fumigatus* with 2.4% NaOCl resulted in an average ( $n = 11$ ) of 94% (range = 92.5% to 95.8%) reduction of spore-eluted allergen concentration at all exposure time intervals (30 sec., 1 min, and 5 min) (Table III). At time zero, the spore-eluted allergen levels of *A. fumigatus* controls from ATCC and reconstituted spore-based antigen controls (Gibson Laboratories) were  $2.89 \times 10^6$  spores/mL and  $7.90 \times 10^6$  spores/mL, respectively. Following exposure to 2.4% NaOCl

**TABLE I. Efficacy of 2.4 Percent NaOCl on Laboratory Mold Stocks**

| Test Organism                      | Carrier Type | Conidia Inoculum  | Dry Carrier Recovery (CFU) | Total Positives per 10 Test Carriers |         |
|------------------------------------|--------------|-------------------|----------------------------|--------------------------------------|---------|
|                                    |              |                   |                            | Batch 1                              | Batch 2 |
| <i>Alternaria alternata</i>        | Non-porous   |                   | $3.2 \times 10^3$          | ND                                   | ND      |
|                                    | Porous       | $2.0 \times 10^5$ | $4.3 \times 10^3$          | ND                                   | ND      |
| <i>Aspergillus niger</i>           | Porous       | $5.5 \times 10^5$ | $2.2 \times 10^5$          | ND                                   | ND      |
| <i>Cladosporium herbarum</i>       | Non-porous   |                   | $1.2 \times 10^4$          | ND                                   | ND      |
|                                    | Porous       | $4.5 \times 10^5$ | $1.2 \times 10^4$          | ND                                   | ND      |
| <i>Mucor bainieri</i>              | Non-porous   |                   | $1.6 \times 10^5$          | ND                                   | ND      |
|                                    | Porous       | $3.5 \times 10^5$ | $1.9 \times 10^5$          | ND                                   | ND      |
| <i>Penicillium chrysogenum</i>     | Non-porous   |                   | $4.2 \times 10^4$          | ND                                   | ND      |
|                                    | Porous       | $6.1 \times 10^5$ | $2.7 \times 10^5$          | ND                                   | ND      |
| <i>Stachybotrys chartarum</i>      | Non-porous   |                   | $1.4 \times 10^4$          | ND                                   | ND      |
|                                    | Porous       | $1.3 \times 10^6$ | $1.3 \times 10^4$          | ND                                   | ND      |
| <i>Trichophyton mentagrophytes</i> | Porous       | $3.0 \times 10^6$ | $3.0 \times 10^6$          | ND                                   | ND      |

Notes: 2.4% NaOCl is the concentration of sodium hypochlorite in common household mold disinfectants.

ND = Not detected; detection limit = 1 CFU.

**TABLE II. Efficacy of 2.4 Percent NaOCl on Household Mold Isolates**

| Mold Species                  | Inoculum <sup>A</sup><br>CFU/tile | Control Tile <sup>B</sup><br>CFU/tile | Replicate<br>Tile #1 | Replicate<br>Tile #2 | Replicate<br>Tile #3 | Replicate<br>Tile #4 |
|-------------------------------|-----------------------------------|---------------------------------------|----------------------|----------------------|----------------------|----------------------|
| <i>Penicillium</i>            | $1.6 \times 10^7$                 | $1.60 \times 10^6$                    | ND                   | ND                   | ND                   | ND                   |
| <i>Rhizopus</i>               | $4.5 \times 10^6$                 | $2.85 \times 10^5$                    | ND                   | ND                   | ND                   | ND                   |
| <i>Aspergillus</i>            | $4.2 \times 10^6$                 | $1.03 \times 10^5$                    | ND                   | ND                   | ND                   |                      |
| <i>Stachybotrys chartarum</i> | $9.4 \times 10^3$                 | $6.0 \times 10^1$                     | ND                   | ND                   | ND                   | ND                   |
| <i>Alternaria</i>             | $1.6 \times 10^3$                 | $6.0 \times 10^1$                     | ND                   | ND                   | ND                   | ND                   |
| <i>Helminthosporium</i>       | $1.1 \times 10^3$                 | $4.0 \times 10^1$                     | ND                   | ND                   | ND                   |                      |

<sup>A</sup>Original inoculum.

<sup>B</sup>Dried inoculum prior to application of disinfectant.

**TABLE III. *A. fumigatus* Spore Eluted Allergen Reduction after Treatment with 2.4% NaOCl**

| <i>A. fumigatus</i> Culture <sup>A</sup> |                |                           |                          | <i>A. fumigatus</i> Antigen Control <sup>B</sup> Experiments | <i>A. fumigatus</i> Spore Eluted Allergen Reduction | Average from Both          |
|------------------------------------------|----------------|---------------------------|--------------------------|--------------------------------------------------------------|-----------------------------------------------------|----------------------------|
| Sample                                   | Treatment Time | Allergen Eluted Spores/mL | Reduction (# Replicates) | Allergen Eluted Spores/mL                                    | Total% Reduction (# Replicates)                     | % Reduction (# Replicates) |
| <i>A. fumigatus</i> <sup>C</sup>         | 0              | $2.89 \times 10^6$        | NA <sup>D</sup>          | $7.90 \times 10^6$                                           | NA                                                  | NA                         |
| 2.4% NaOCl                               | 30 sec         | $1.32 \times 10^5$        | 95.4%<br>(n = 1)         | $3.12 \times 10^5$                                           | 96%<br>(n = 2)                                      | 95.8%<br>(n = 3)           |
| 2.4% NaOCl                               | 1 min          | $2.16 \times 10^5$        | 92.5%<br>(n = 2)         | $4.32 \times 10^5$                                           | 94.5%<br>(n = 2)                                    | 93.5<br>(n = 4)            |
| 2.4% NaOCl                               | 5 min          | $1.46 \times 10^5$        | 94.9%<br>(n = 2)         | $3.46 \times 10^5$                                           | 95.6%<br>(n = 2)                                    | 95.3<br>(n = 4)            |

<sup>A</sup>ATCC-purchased culture.

<sup>B</sup>Reconstituted spore-based antigen control purchased from Gibson Laboratories.

<sup>C</sup>*A. fumigatus* control sample prepared in inactivated, pH neutralized 2.4% sodium hypochlorite disinfectant.

<sup>D</sup>Not applicable.

for 30 sec, spore-eluted allergen levels were reduced to  $1.32 \times 10^5$  and  $3.12 \times 10^5$ , corresponding to a 95.4% and 96% reduction in spores eluting allergen, respectively. After 1-min exposure to disinfectant, spore eluted allergen levels were reduced by an average ( $n = 2$ ) of 92.5% ( $2.16 \times 10^5$  spores/mL) in the *A. fumigatus* culture from ATCC and 94.5% ( $4.32 \times 10^5$  spores/mL) in the reconstituted spore-based antigen controls from the original allergen level ( $2.89 \times 10^6$  spores/mL). Five-minute exposures to disinfectant resulted in an average ( $n = 2$ ) 94.9% ( $1.46 \times 10^5$ ) and 95.6% ( $3.46 \times 10^5$ ) reduction in the ATCC culture and the reconstituted spore-based antigen control, respectively. Results indicate rapid (30 sec) reduction of allergenicity as measured by immunostaining of spore-eluted allergen following 2.4% NaOCl treatment.

## DISCUSSION

Mold types targeted in this study are commonly found as indoor contaminants associated with symptoms of allergy (i.e., upper respiratory tract symptoms, coughing, wheezing) and asthma. Less certain are the health effects associated with mold toxin-production (i.e., *Stachybotrys*, *Aspergillus*, *Penicillium*) that are suspected to cause a wide range of health effects including immunosuppression, organ toxicity, cancer, and more.

Although recent studies have examined house dust or indoor air quality with respect to fungal contaminants,<sup>(32,33)</sup> little information is published related to the occurrence of indoor mold contamination on surfaces throughout the home environment that may also contribute to exposure.<sup>(34)</sup> This study shows that contact with mold in indoor environments is common and that potentially harmful mold is frequently present on a wide variety of indoor household surfaces, including unexpected sites that may not be targeted for routine cleaning and disinfecting. Guidelines for mold abatement generally stress reduction of moisture to prevent mold growth. However, certain household microenvironments (i.e., showers) are chronically moist and require additional safeguards to minimize mold growth indoors.

Mold types targeted for our study were selected based on reports of common prevalence indoors and/or association with human allergenic response. Previous studies found that the southwest, west, and southeast regions of the United States harbor the highest mold counts in indoor air samples from buildings.<sup>(35)</sup> While our study included these same geographical regions, no attempt was made to analyze the geographical variability of the samples. All samples were collected during June and August, due to high counts expected during the summer months.<sup>(35)</sup> Like the study of indoor air by Shelton et al.,<sup>(35)</sup> *Cladosporium* and *Aspergillus* or *Penicillium* were also identified as the most common molds on indoor surfaces, while *Stachybotrys* was not highly prevalent (1.2% positive). Known to be a mold that is more commonly isolated from porous surfaces, often in hidden areas with high (>93%) relative humidity or following flood or leakage events, it was not surprising to see a low prevalence of *Stachybotrys* in

our study. Surfaces were frequently contaminated with "other molds" (52.7%; 575/1092 positive). Isolates were recorded as "other mold" if morphological characteristics were not indicative of a definitively identifiable mold type from our target list. Surfaces were also frequently contaminated with hyphal fragments (33.2%; 363/1092) that could not be identified due to the presence of incomplete structures. Recently, studies have shown that hyphal fragments are readily aerosolized in indoor environments, cause significant immunological reactivity, and should be considered in indoor air quality assessments.<sup>(20,36)</sup> Therefore, hyphal fragments should not be discounted in future environmental mold surveys.

The contact sampling method (i.e., tape-lift sampling and microscopy) provided qualitative information on both the culturable and nonculturable (i.e., hyphal fragments) fractions of molds present and was useful for the collection of a wide variety of mold types. In addition, this method of direct examination provides a non-technical and inexpensive mold test that untrained personnel can perform quickly and easily. While the potential variability of sampling technique used by the volunteers in the study could not be controlled, the direct sampling method was useful for relative measurement of mold types in various environments and aided in the identification of indoor reservoirs for molds settled on, and potentially colonizing, surfaces. A disadvantage to the tape-lift sampling method is that some molds are difficult to differentiate based on morphology alone, i.e., *Aspergillus* and *Penicillium*, which were generally grouped together in our study. In addition, the methods of this study were selective for specific target areas and mold types common to those test areas and did not necessarily represent the total indoor mold diversity or concentration.

Despite the high relative humidity in the shower environment, these indoor microenvironments were the least frequently positive for mold, possibly because they are often targeted for mold abatement using cleaners and disinfectants. Although moisture reduction is recommended as the first step to reduce indoor mold colonization, our study suggests that the use of 2.4% NaOCl disinfectants would also have an impact on the presence of culturable mold and mold allergens, even in chronically moist environments. In addition, 2.4% NaOCl disinfectants are expected to reduce mold counts and allergens in environments not usually targeted for disinfecting, such as windowsills and refrigerator seals, which are also prone to moisture and high humidity.

The results of this study support those of others concluding that hypochlorite disinfectants are effective antimicrobials against mold.<sup>(37)</sup> In the current study, *Trichophyton mentagrophytes* was not culturable in 10/10 trials following 10-min exposures to 2.4% NaOCl on porous surfaces (Table I). This agrees with previous research in which *T. mentagrophytes*, *T. raubitschekii*, and *T. tonsurans* were readily inactivated by 1% chlorine.<sup>(38)</sup> To remove mold from non-porous surfaces, the U.S. Environmental Protection Agency advises wiping or scrubbing mold-contaminated sites with water or water and detergent.<sup>(26)</sup> However, a preliminary field study shows

that cleaning household showers with liquid soap and water reduced culturable mold counts by 1-log<sub>10</sub>, compared with a >4-log<sub>10</sub> reduction following use of 2.4% NaOCl disinfectants (data not shown). Further evidence of the efficacy of sodium hypochlorite as an antifungal disinfectant is the fact that 0.04% NaOCl has been recommended to inactivate fungi on the surface of grains, nuts, and vegetables, when there is interest in the detection of these organisms in the interior of these products.<sup>(21)</sup>

Previous studies have shown that even when mold spores are no longer culturable, they may still illicit an allergic response due to intact surface antigens.<sup>(39)</sup> This fact, in part, has prevented state and federal health agencies from recommending the use of biocides for mold abatement.<sup>(28)</sup> Based on our ELISA antigen results, 2.4% NaOCl reduced the level of spore eluted allergenic proteins present in *A. fumigatus* by 92.5–96.0%. These results are consistent with previous studies on bleach fragmentation of *A. fumigatus* and other household allergens.<sup>(22,23)</sup> The minimum detection limit for the ELISA test ranged from  $1.5 \times 10^5$  to  $3.1 \times 10^5$  spores/mL. Based on this detection limit, most of the disinfected *A. fumigatus* samples were analyzed at the lower limit of detection ( $10^5$  spores/mL), and thus, we could not measure reductions >96–98%. Method sensitivity was slightly higher for the *A. fumigatus* antigen control stock from Gibson Laboratories because starting spore concentrations were higher in these samples. While additional allergen fragmentation studies are needed on a wider variety of mold types, currently, a large data gap is the identification of indoor mold exposures and associative adverse health outcomes. Recently, researchers used epidemiologic approaches to evaluate the impact of home cleaning and disinfectants on the quality of life for asthmatic residents.<sup>(40,41)</sup> Significant improvements in health were reported, but additional studies are needed to confirm the significance of the relationship between improved health and the use of bleach-based disinfectants in the home.

## RECOMMENDATIONS

Following exposure to mold, symptom manifestation and severity depends largely on host response/susceptibility, dose, exposure route, time and frequency of the exposure, and nature or virulence of the organism. While clinical trials and causal relationships proving the relationship between indoor mold presence and human disease are lacking, many studies in the literature show an association with asthma and allergic disease.<sup>(11–14,39)</sup> Risks may be acute, chronic, or asymptomatic, yet progressive, where the presence of allergic disease to a specific agent can increase on re-exposure to that agent.<sup>(42)</sup>

Little is known about the prevalence of mold in home environments or the long-term effects of chronic mold exposures; however, abatement procedures are recommended. Contact with low concentrations (2.4%) of NaOCl (i.e., concentrations common to commercial household products) for 5–10 min reduces the concentration of spore eluted allergen levels by >95% and result in a >5-log<sub>10</sub> reduction of culturable molds

on surfaces, respectively. Therefore, in addition to moisture control efforts, 2.4% NaOCl treatment of hard surfaces should also be recommended as a means for reduction of indoor mold proliferation and allergen levels, particularly in chronically moist indoor microenvironments. The overall health benefit of biocide use, however, needs to be quantitatively evaluated. Future epidemiologic studies should also include simultaneous efforts to validate less expensive tools of environmental health monitoring, such as the ELISA methods used in this study.

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