Laboratory and Field Evaluation of a New Methodology for the Sampling and Analysis of Fungal Fragments

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Indoor exposures to molds contribute to occupant respiratory disease and symptoms (IOM, 2004).

However, previous studies have not been able to establish a clear cause-and-effect relationship between inhalation of fungal particles and adverse respiratory effects.

- No significant association between dampness or visible mold and the spore concentration level (Garrett et al., 1998; Chew et al., 2003).
Background

- Exposures to submicrometer-sized fungal particles (i.e., fungal fragments) potentially contribute to adverse health effects due to: their (i) small physical size; (ii) composition - fungal antigens (Górny et al., 2002) and mycotoxins (Brasel et al., 2005).

- The contribution of these fungal fragments to exposure and adverse health outcomes are poorly characterized.
Background (cont’d)

- Assessing exposures to these fungal fragments for health effect studies is challenging due to:
  - Lack of a proper sampling method;
    - The difficulty in collecting (the particle bounce problem)
  - Lack of the proper analyses:

  Traditional analytic methods such as culture-based method and microscopic method may not be suitable for analysis of submicron fungal particles.
(1-3)-β-D-glucan has been used as a biomarker of fungal biomass (Chew et al., 2001; Rao et al., 2004).

- Beta-glucan and chitin: shape and rigidity of the cell wall structure.
- (1-3)-β-D linkage is mainly related to fungi.
- (1-3)-β-D-glucan: up to 60% of the dry weight of the cell wall of fungi (Douwes 2005).
Objectives

- To develop and test a new sampling system for collecting submicrometer-sized fungal particles:
  - To determine the feasibility of (1-3)-β-D-glucan assay for quantifying submicrometer-sized fungal particles.

- To collect pilot data in moldy houses.
To develop and optimize the new sampling system.

To determine the feasibility of (1-3)-β-D-glucan assay.
METHODS AND MATERIALS

Experimental set-up

Aerosolized Fungal particles

OPC-1

> 2.5 um

PM<sub>2.5</sub>

OPC-2

1.0 - 2.5 um

OPC-1

Filter

CNC
Testing and optimization

- Penetration test: monodisperse PSL particles (0.54, 1.79, and 3.94 μm).

\[
\text{Penetration rate (\%)} = \frac{N_{\text{downstream}}}{N_{\text{upstream}}} \times 100
\]

- Bounce test with fungi
  - Microorganisms: *Aspergillus versicolor* and *Stachybotrys chartarum*.
  - Growth media: 1-week malt extract agar (MEA).
Feasibility test of (1-3)-β-D-glucan assay

- (1-3)-β-D-glucan assay:

  The kinetic chromogenic *Limulus* Amebocyte Lysate (**LAL**) assay.

- Procedure

  (i) Sample collection; (ii) Sample extraction; (iii) Take an aliquot (0.5 ml)
  (iv) **LAL** assay.
**Results**

Penetration curves and the $D_{50}$ values

![Graph showing penetration curves for different optical diameters](image)

- **PM2.5 Cyclone**
- **PM1.0 Cyclone**

Diameters for various types:
- **Ascospores (3.7-7.5)**
- **Aspergillus/ Penicillium (2.6-4.8)**
- **Cladosporium (5.2-10.9)**
- **Alternaria (11.2-17.8)**
- **Stachybotrys (5.0-7.0)**

Penetration rate (%): 100, 50, 0
Results (cont’d)

Experiments on spore bounce

Total spores collected onto after-filter as estimated by microscope (spores)

- S. chartarum
- A. versicolor

Detection Limit (55)

Total particle numbers entering the collection system as measured by the OPC-1 (particles)
RESULTS (cont’d)

**Airborne (1-3)-β-D-glucan concentration of fragment and spore size fractions**

![Graph showing airborne (1-3)-β-D-glucan concentration of fragment and spore size fractions for A. versicolor and S. chartarum.](image-url)
The Field Test

- **New Orleans** (3 homes) and Southern Ohio (2 homes).

- **Sampling seasons**
  - **Summer** (June ~ September 2006).
Sampling and measuring the (1-3)-β-D-glucan

- **Button sampling** (FIG. 1) and sampling of fragment sized particles (FIG. 2).
- LAL assay.

![FIG. 1. Button Inhalable sampler](image1)

![FIG. 2. Sampling box for fragment sized particles](image2)
Methods and Materials (cont’d)

Photos

<table>
<thead>
<tr>
<th>(A) Installed in indoor</th>
<th>(B) Installed in outdoor</th>
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<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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Picture 1. Field experimental Sep-up installed in: (A) indoor; and (B) outdoor air
The Fragment Collection System (FCS) vs. the Button Sampler

\[ R = 0.919 \]
\[ (P < 0.0001) \]
Results (cont’d)

Airborne (1-3)-β-D-glucan Concentrations in Field Samples

![Graph showing airborne (1-3)-β-D-glucan concentrations in summer and winter samples.](image-url)
**RESULTS** (cont’d)

**Fragment/spore Ratio in Field Samples**

- **Laboratory test**

<table>
<thead>
<tr>
<th></th>
<th>A. versicolor</th>
<th>S. chartarum</th>
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</thead>
<tbody>
<tr>
<td>Summer</td>
<td>-1.00</td>
<td>-1.00</td>
</tr>
<tr>
<td>Winter</td>
<td>0.00</td>
<td>0.00</td>
</tr>
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</table>
Conclusions

- Laboratory evaluation showed that fungal fragments can be successfully separated from intact spores.

- The (1-3)-β-D-glucan assay proved to be sensitive enough for the analysis of all tested fungal aerosols including submicron fungal fragments in size-fractioned samples.

- The good agreement between the measurements of the Button and FCS shows the capability of the Fragment Collection System combined with (1-3)-β-D-glucan assay for quantifying fungal particles in fragment and spore size fraction in real field conditions.
Conclusions

- The results of field sampling showed that the fragment size fraction contained considerable amount of (1-3)-β-D-glucan, which was comparable to the amount measured in the spore size fraction in many situations.

- This is inhalable size-selective sampling method.

- The new methodology is a promising tool for collection and analysis of fragments.
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Any Questions?
Specific Aim 2.

- **Biological assay: β-glucan assay.**

  - Cascade of LAL Assay.

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Sample (β-glucan) + Amebocyte

Activated Factor G

Inactive clotting Enzyme

Activation

Active Clotting Enzyme

Coagulogen (Substrate)

Detecting

Coagulin (Clot)
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