Concentrations of Airborne Asp/Pen Spores in Hospitals as Measured by QPCR and Spore Counts

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Objective

Develop a rapid, sensitive, cost-effective sampling protocol for detecting very low concentrations of *Aspergillus* in highly filtered air

One protocol suitable for:
- Baseline sampling,
- Incident response investigations,
- Post-remediation verification sampling
Sampling Protocol

- Collecting duplicate samples using the Bi-Air Filter Cassette
- 100% of the first sample was analyzed by microscopy at 600X magnification
- Second sample was analyzed by QPCR only if *Asp/Pen* spores were detected in the first sample and risk assessment needed

*QPCR: Quantitative Polymerase Chain Reaction*
Bi-Air Filter Cassette
Bi-Air Filter Cassette: 25 mm MCE Filter

Four air inlets
Duplicate Samples: Both Total Spores **and** Cultures or QPCR
Bi-Air Sample Trace

- Sample Trace < 2% of total filter area
- No filter breakage
- Preparing QPCR samples
- Smaller wash volume
- Greater sensitivity for QPCR
Spore Counts by Microscopy

v.

Spore Equivalents by QPCR
QPCR on Filter Media

Filters are “sterile”,
And free of spores by microscopy,
But not free of “spore equivalents”
as measured by QPCR

Abstract 272: Park & Shogren
25 mm MCE Filter Blanks
Analyzed by QPCR

<table>
<thead>
<tr>
<th>FILTER</th>
<th>0</th>
<th>0</th>
<th>3</th>
<th>37</th>
<th>156</th>
</tr>
</thead>
</table>

6 blank MCE filters
Data are Total Sp-Eq / filter
Avg = 37 Sp-Eq / filter
Range & Average would be “unacceptable”
QPCR Analysis Using The BA Cassette

6 Blank MCE Filters: Sp-Eq/Trace

<table>
<thead>
<tr>
<th>TRACE</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>6.2</td>
<td></td>
</tr>
</tbody>
</table>

Avg = 1.4

BA Sample < 4% of Filter

Sp-eq/Trace
## Filter Size Matters

**Expected average Sp-Eq on MCE Filters**

<table>
<thead>
<tr>
<th>FILTER MEDIA</th>
<th>AVERAGE SP EQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bi-Air Trace</td>
<td>1.4</td>
</tr>
<tr>
<td>25 mm</td>
<td>37.2</td>
</tr>
<tr>
<td>37 mm</td>
<td>81.6</td>
</tr>
</tbody>
</table>
Characteristics of QPCR
QPCR Measures “Spore Equivalents”
Example 168 Liter Sample

<table>
<thead>
<tr>
<th>TYPE</th>
<th>SPORES</th>
<th>SP/M³</th>
<th>SP-EQ</th>
<th>EQ/M³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scopulariopsis</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td><em>P. chrysogenum</em></td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td><em>A. versicolor</em></td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>38</td>
</tr>
</tbody>
</table>

Correlations: Based on Spores & Spore Equivalents per sample, not per m³
Commissioning 13 New Operating Rooms: *Asp/Pen* Concentrations in Four OR’s

<table>
<thead>
<tr>
<th>SPORES</th>
<th>SP/M³</th>
<th>SP EQ*</th>
<th>EQ/M³</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>31</td>
<td>220</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>7</td>
<td>74</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>7</td>
<td>65</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>4</td>
<td>60</td>
</tr>
</tbody>
</table>

*“Common” Asp and Pen

Pass or Fail these 4 OR’s?
Only $\approx 10\% \Rightarrow$ QPCR

Rank Order of Sample Results
QPCR: Asp & Pen on MCE Filters

½ of Filter Analyzed
Characterizing Hospital Environments
Airborne Fungi Identified by QPCR in Hospital Samples

- A. penicillioides
- A. niger
- Eurotium amstel
- A. sydowii
- A. ustus
- A. flavus
- A. versicolor

- P. chrysogenum
- Scopulariopsis char
- P. corylophilum
- P. variabile

Rank Order
Method Sensitivity v. Problem Detection

Problem Operating Room

- Surgeon complaints
- 10-min Air-O-Cell sample
  - “No problem”
- 3-hour Bi-Air sample
  - 4 Asp/Pen spores [25 spores/m³]
  - One Asp/Pen spore detected every 45 minutes
  - One Stachybotrys spore

Recommendation: inspection by facilities
Result: Two walls remediated
### Baseline Concentrations of Airborne *Asp/Pen* Spores

<table>
<thead>
<tr>
<th>Spores/m³</th>
<th>OR’s</th>
<th>ICU’s</th>
<th>Non Critical Areas</th>
<th>Surgical Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>20</td>
<td>29</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>Max</td>
<td>6*</td>
<td>30</td>
<td>222</td>
<td>78</td>
</tr>
<tr>
<td>16th %-tile</td>
<td>1.7</td>
<td>2.3</td>
<td>3</td>
<td>4.4</td>
</tr>
<tr>
<td>50th %-tile</td>
<td>2.1</td>
<td>5.2</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>84th %-tile</td>
<td>2.6</td>
<td>11.5</td>
<td>37</td>
<td>43</td>
</tr>
</tbody>
</table>

*Minimum LOD necessary to assess “abnormal conditions”*
Example: Clearance of an ICU Ward
Referenced Guidance

Healthcare Infection Control Practices Advisory Committee (HICPAC); 2003

“… aspergillosis cases have occurred when fungal spore concentrations in Positive Environment air ranged as low as 0.9 cfu/m³…”

Clearance criteria adopted:

- < 0.8 Asp/Pen spores/m³ [< 0.9 spores/m³]

Required to achieve this LOD:

- 9 [5 min] N6 culturable samples at 28 lpm
- 9 [10 min] Air-O-Cell samples at 15 lpm
- 1 [7 hour] Bi-Air sample at 3 lpm
ICU Clearance

- 13 locations sampled
- 7-hour BA samples at 3 lpm
- Volume = 1,260 liter
- LOD = 0.8 spores/m³
- Microscope on-site
- Detect *Asp/Pen* spores
- QPCR not required
- One room failed:
  - One spore in a 7-hour sample
  - Re-cleaned, then passed

Total Spores are a more conservative criterion than cfu’s
- Spores/cfu undefined
- If collecting 5-minute N6 or 10-minute Air-O-Cell samples:
  - 126 samples to achieve the same LOD as 14 BA samples [lower cost]
Advantages of Bi-Air Protocol

- Single, simple methodology
  - Baseline surveys
  - Incident investigations
  - “Clearance” samplings

- Rapid Exposure Assessment
  - Microscopic analysis on first sample
  - Large sample volumes > 1,000 liters
  - Limit of Detection of 0.9 spores/m³ or less
  - Long sample times of 3 – 8 hours
  - Minimize false negatives
  - [Collecting 4 spores in 3 hours = detecting a “rare event”]
Advantages of Bi-Air Protocol

- Adequate LOD achieved with one BA sample
  - One BA v. 9 AOC or N6 samples per sample location

- Rapid Risk Assessment
  - QPCR analysis of second sample
  - Infection Control

- Small BA sample trace < 4 % of total filter area
  - Contaminant spores by QPCR minimized
Characterizing Hospital Environments

Objective
Rapid, sensitive, cost-effective protocol suitable for detecting *Aspergillus* in highly filtered air

Sampling method
Duplicate samples using the Bi-Air filter cassette

Method of analysis
Exposure Assessment - Microscopy
Risk Assessment – Confirmed by QPCR
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