Rapid, Economical, and Population-Protective Monitoring of Mold Disturbances in Hospitals and other Sensitive Environments.

AIHce 2005
May 23, 2005
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Overview of Presentation

Introduction to Real Time PCR
The Problem
The Solution
- An Economical Approach to PCR: The Method
Case Studies
Summary

Q-PCR The Promise

- Does not require heavily trained mycologists for the identification
- Is an instrumental method, far more conducive to QA Procedures, traceability and defensibility
- Speciation of Penicillium/Aspergillus can be accomplished in a day
- Since loading of samples is not an issue as it is in direct microscopy methods, air sampling constraints are lifted
- Identification of a single selected species of interest is possible

Q-PCR - Determining Validity and Use

- Initial validation with EPA in 2002
  - Basic validation on Cladosporium species
- Initial In-house study in 2003
  - Basic Method Comparisons of Culturable, Non-Culturable and PCR
- Second In-house study in 2003-04
  - Precision and Accuracy Studies
- Optimization and Use by FA in 2004-05
  - Case Studies

Concerns in Hospitals

- Primary interest lies in the prevention of Aspergillosis disease related to mold disturbances in healthcare environments.
- Monitoring will be done during/after mold disturbances.
- Interested in:
  - Quantification of species of concern
  - Rapid turnaround time
  - Economical method

The Other Methods:

The Problems
### Viable
- Require 7 - 10 days to grow
- May not grow at all.
- Impractical

### Non-Viable
- Rapid method.
- Unable to differentiate among Pen/Asp species.
- Cannot quantify species that cause Nosocomial infection.

### Q-PCR
- Have to pick what I want to look for, otherwise it can get costly
- Spore equivalents based on all genetic material, not just spores
- Optical microscopy and molecular genetics differ taxonomically

### The Solution
Utilize combinations of non-viable and quantitative PCR analytical methods in a process that provides the most relevant results rapidly and economically.

### Mold Investigation
- Visual Inspection:
  - Mold Growth
  - Dust accumulation
- To decide where to sample for what has been/will be disturbed.

**The Method:**
An Economical Approach to PCR
Collect Tape Lift and Bulk samples of mold growth

- Analyze the tape lift sample(s) utilizing non-viable technique.

- Based on non-viable tape lift results, select a focused panel of species and analyze bulk samples of mold growth or accumulated dust by PCR
  - An experienced microbiologist will be able to make suggestions when identifying Pen/Asp species

**Non-viable Tape Lift**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Result</th>
<th>PCR Bulk Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tape Lift</td>
<td>Non-viable</td>
<td>PCR Bulk Result</td>
</tr>
<tr>
<td>Tape Lift</td>
<td>PCR Result</td>
<td>PCR Bulk Result</td>
</tr>
</tbody>
</table>

**PCR Bulk Results**

Based on non-viable tape lift and PCR bulk results, select “marker” specie(s) to be monitored during construction/remediation/exposure assessment.

- Aspergillus
- Chaetomium
- Penicillium
- Stachybotrys

Choosing “Marker” specie(s) will also allow for the monitoring of the mold disturbances using the best (specific and economical) method(s) of analysis
- Non-viable airs
- PCR airs
- Both

- PCR with selected few species such as A. fumigatus and P. chrysogenum can be used.
- To save money, NVA with Stachybotrys species can be used
- But PCR can also be used with all 3 species.
Case Studies

AIHce 2005: Economical use of PCR

Q-PCR – Case 1: Hospital Setting

- Concerns during construction for:
  - Disturbance of mold growth.
  - Disturbance of accumulated dust.
  - Nosocomial infections in immunocompromised individuals.

- Monitor the disturbance using the best fit method(s)
  - NVA, PCR, or both

Q-PCR – Nosocomial Infections

- Aspergillosis - large spectrum of respiratory diseases caused by members of the genus Aspergillus
  - In immunocompromised individuals, invasive pulmonary infection, which may disseminate to other organs, including brain, skin and bone
  - Focus on preventing airborne exposure to various Aspergillus species.
  - PCR Panel for Preventing Aspergillosis*
    - Aspergillus flavus, fumigatus, terreus, versicolor, niger

*Other species may be added into panel based on facility’s infection history

Case 1 Finding: Hospital Exterior

- Visible water intrusion and mold growth found at exterior drywall of hospital building.
- Stachybotrys was the predominant species found in the mold growth.
- Non-Viable Air (NVA) analysis utilizing Stachybotrys as the marker was used for air clearance.

NV Tape Lift

PCR Bulk
Case 1 Finding: Hospital Interior

- Visible mold discovered on wallboard behind wainscot of hospital corridor.
- Took tape lift and bulk samples of mold growth.
- PCR panel chosen for bulk sample analysis based on tape lift results.
- Nosocomial panel also used due to hospital setting.

 NV Tape Lift           PCR Bulk

Clearance - PCR-air

Q-PCR - Case 2: Biotech Setting

- Potable water leak
- No visible areas of mold growth on interior surfaces, however moisture testing led to a DT plan involving both wet and dry areas for comparison.
- After destructive investigation and moisture tests of space - Identified six areas with localized mold growth.
- Selection of panels in manner previously described.

Non-Loss Related

Loss Related
Q-PCR - Case 2 Findings and Decisions

- Using PCR-bulk the key indicator mold was found to be *P. chrysogenum*.
- Prior to remediation, affected and unaffected areas were characterized by NVA and PCR-air.
- Monitored remediation using PCR-air and indicator mold - *P. chrysogenum*.
- During remediation, other water leaks and mold sources identified, and species added to panel.
- Post-remediation, clearance of room using PCR-air - *P. chrysogenum* and other selected species used.
- PCR used throughout, for both the differentiation and long term (8-hr) sampling benefits.

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Clearance - PCR-air

- Outdoors - Very High
- ICU rooms - Very Low
- ICU corridor Med/Low
- Hospt. corridor - High
- ER - Medium
- Door to ICU
- Door to ER
- Door to outdoors

Levels of *A. Fumigatus*

** Patient Location

Q-PCR - Case 3 - The Patient Dies

- Of Aspergillosis - *A. Fumigatus* identified as the cause.
- No visible mold growth found in the patient’s room.
- However hospital focus is immediately on the room - Plans for quick isolation and demolition.
- A more extensive evaluation of airborne levels by PCR is undertaken for *A. fumigatus* with 24hr turnaround time.
- Pattern clearly indicated that airborne levels of *A. Fumigatus* highest outdoors and progressively lower as one moves to patient wings and rooms.
- Airborne levels suggest an entirely different course of action.

Summary: PCR Method

- Quick
- Specific
- Economical
  - Combining non-viable & PCR methods of bulk analysis
  - Choosing marker species for PCR air sampling analysis
Thank You!

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Acknowledgements:
Terri Chen, MPH, Dan Cox, PhD, CIH, Melissa Piercey, MSc,
John Martinelli, CMC, CIAQM, Pete Kaminski