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

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Sampling Protocol

- Collect duplicate samples using the Bi-Air Filter Cassette
- Analyze 100 % of the first sample by microscopy at 600X magnification
- Analyze second sample by QPCR* only if *Asp/Pen* like spores were detected in the first sample

*QPCR: Quantitative Polymerase Chain Reaction

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Bi-Air Filter Cassette: 25 mm MCE Filter

Four air inlets

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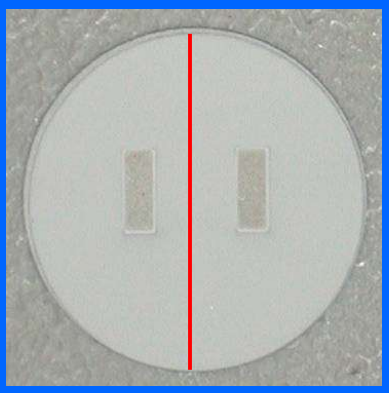
Duplicate Samples: Both Total Spores and Cultures or QPCR



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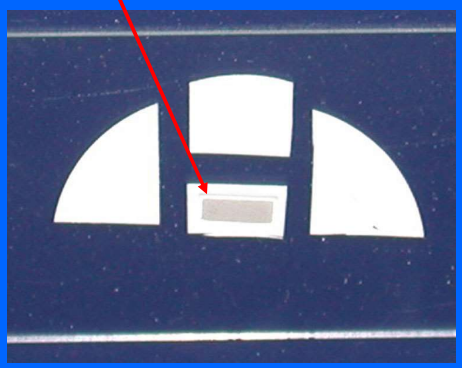
Bi-Air Sample Trace

Sample Trace < 2 % of total filter area



- No filter breakage
  - Preparing QPCR sample
- Smaller wash volume
  - Greater sensitivity
- < 4 % of filter analyzed
  - Fewer background spores

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Spore Counts by Microscopy

v.

Spore Equivalents by QPCR

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QPCR Measures "Spore Equivalents"

Example 168 Liter Sample

TYPE	SPORES	SP/M^3	SP-EQ	EQ/M^3
Scopulariopsis	1	6	1	3
A flavus	1	6	1	6
P chrysogenum	1	6	1	27
A versicolor	1	6	1	38

Correlations: Based on Spores & Spore Equivalents per sample, not per m^3

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Commissioning 13 New Operating Rooms: *Asp/Pen* Concentrations in Four OR's

SPORES	SP/M³	SP EQ*	EQ/M³
0	0	31	220
0	0	7	74
1	6	7	65
0	0	4	60

*"Common" *Asp* and *Pen*

Pass or Fail these 4 OR's ?

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Filters Are "Sterile"

[But Not Free of Spore-Equivalents by QPCR]

6 Blank 25 mm MCE Filters

Sp Eq/ [FILTER]	Sp Eq/ [Bi-Air]
0	0
0	0
3	0.1
27	1.1
37	1.5
156	6.2

FILTER MEDIA	AVERAGE SP EQ
Bi-Air Trace	1.4
25 mm	37.2
37 mm	81.6

Sp Eq = "0" in 16 of 42 QPCR samples

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Correlations between QPCR and Microscopy for *Aspergillus* and *Penicillium* Spores

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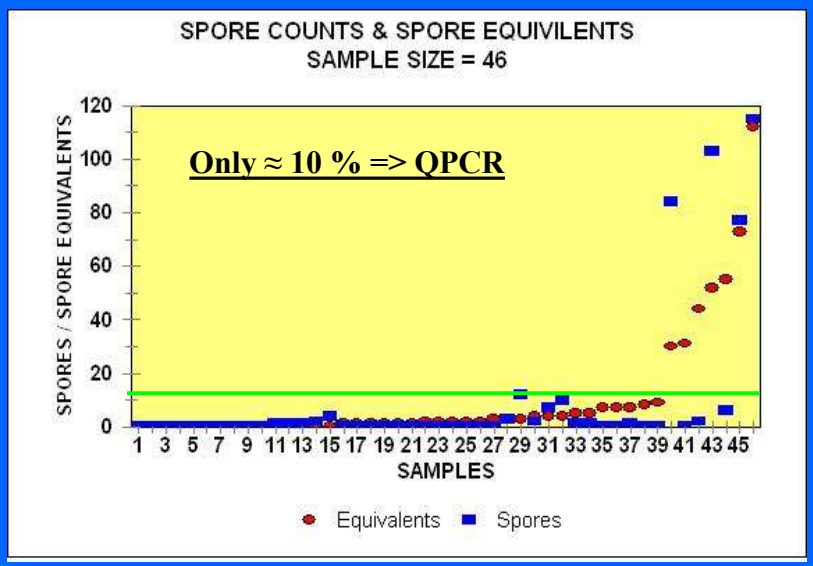
Rank Order of Sample Results



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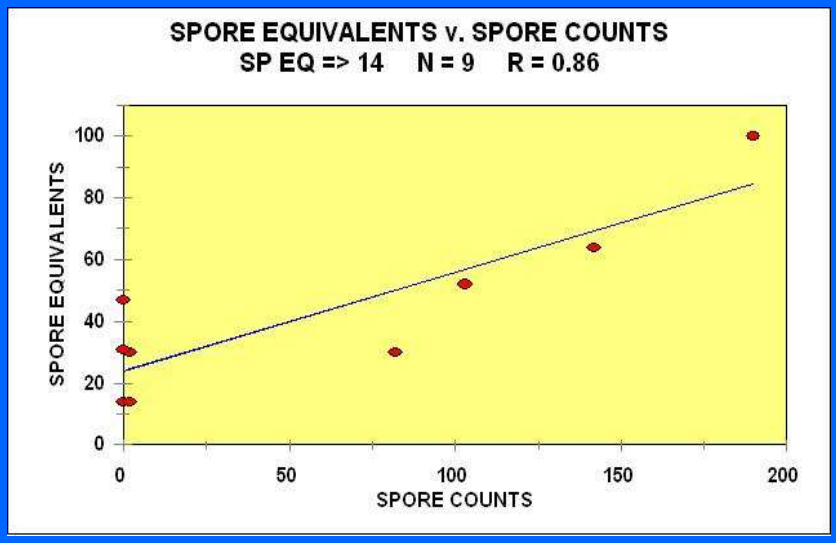
Correlation: QPCR v. Microscopy



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Characterizing Hospital Environments

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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

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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/m^3]**
      - **One *Asp/Pen* spore detected every 45 minutes**
    - **One *Stachybotrys* spore**
  - **Recommendation: inspection by facilities**
  - **Result: Two walls remediated**

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Baseline Concentrations of Airborne *Asp/Pen* Spores

Spores/m^3	OR's	ICU's	Non Critical Areas	Surgical Support
Samples	20	29	25	8
Max	6*	30	222	78
16th %-tile	1.7	2.3	3	4.4
50th %-tile	2.1	5.2	11	14
84th %-tile	2.6	11.5	37	43

*Minimum LOD necessary to assess "abnormal conditions"

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Example: Clearance of an ICU Ward

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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/m^3…"
- Clearance criteria adopted:
  - < 0.8 *Asp/Pen* spores/m^3 [< 0.9 spores/m^3]
- 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

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ICU Clearance

- 13 locations sampled
- 7-hour BA samples at 3 lpm
- Volume = 1,260 liter
- LOD = 0.8 spores/m^3
- 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]

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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/m^3 or less
- Long sample times of 3 – 8 hours
  - Minimize false negatives
  - [Collecting 4 spores in 3 hours = detecting a "rare event"]

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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

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Advantages of Bi-Air Protocol

- BA collects duplicate sample traces
  - Cost effective
  - QPCR only required for those samples in which *Asp/Pen* like spores were detected by microscopy
  - Archive second sample trace for reference

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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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