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

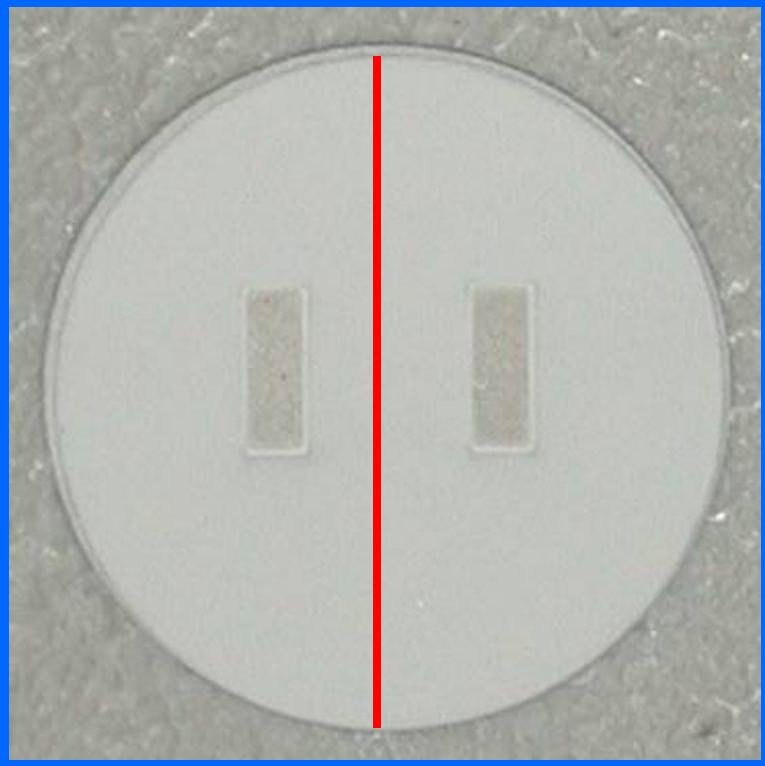
*QPCR: Quantitative Polymerase Chain Reaction

Bi-Air Filter Cassette

Bi-Air Filter Cassette: 25 mm MCE Filter

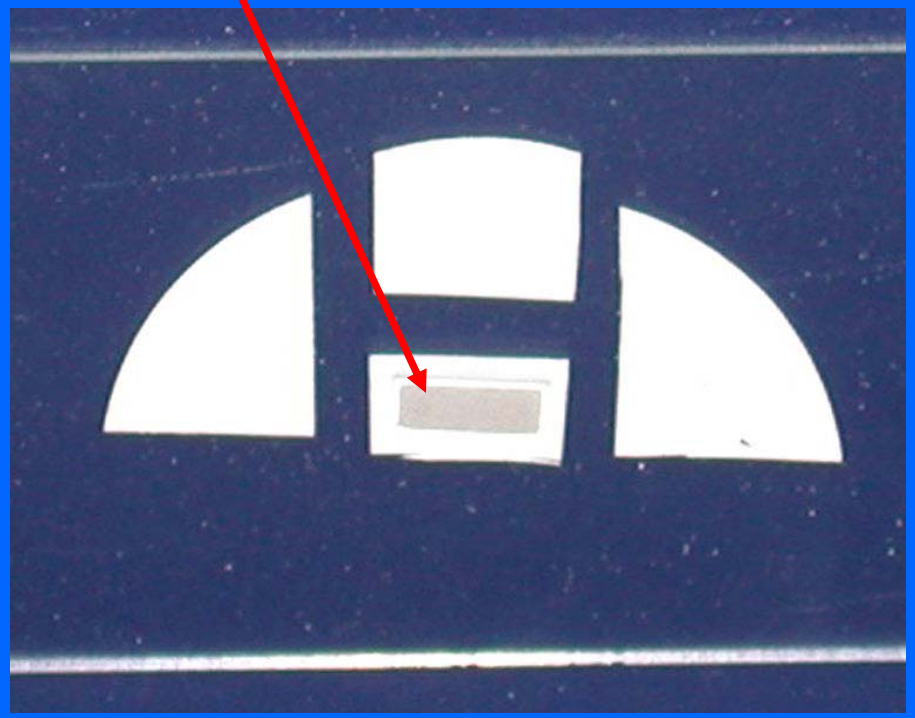
Four air inlets

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

FILTER
0
0
3
27
37
156

6 blank MCE filters

Data are Total Sp-Eq / filter

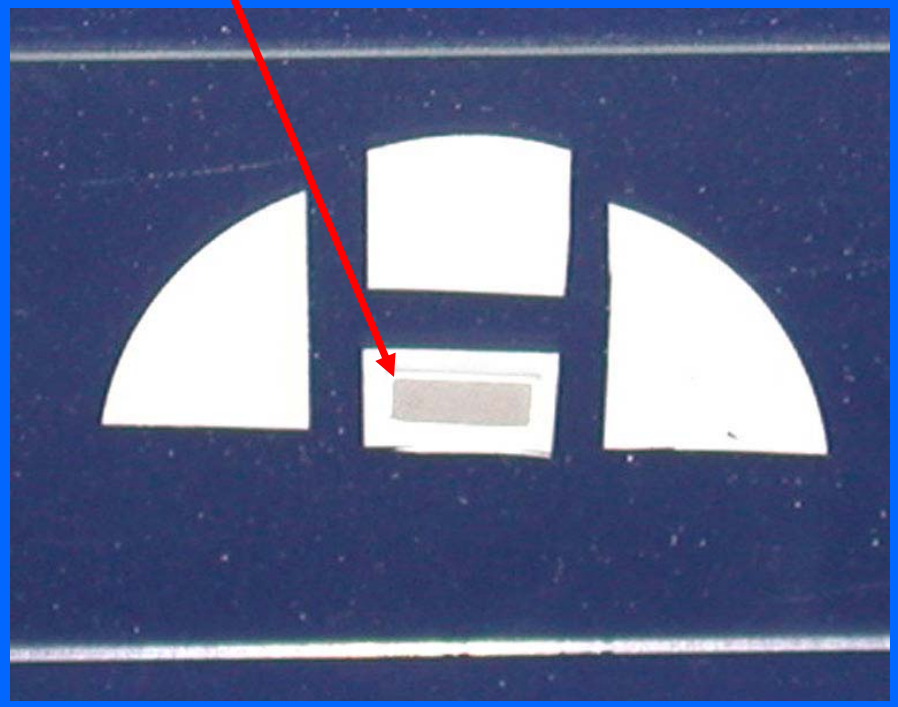
Avg = 37 Sp-Eq / filter

Range & Average would be "unacceptable"

QPCR Analysis Using The BA Cassette

6 Blank MCE Filters: Sp-Eq/Trace

BA Sample < 4% of Filter



TRACE
0
0
0.1
1.1
1.5
6.2

Avg = 1.4

Sp-eq/Trace

Filter Size Matters

Expected average Sp-Eq on MCE Filters

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

Characteristics of QPCR

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

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

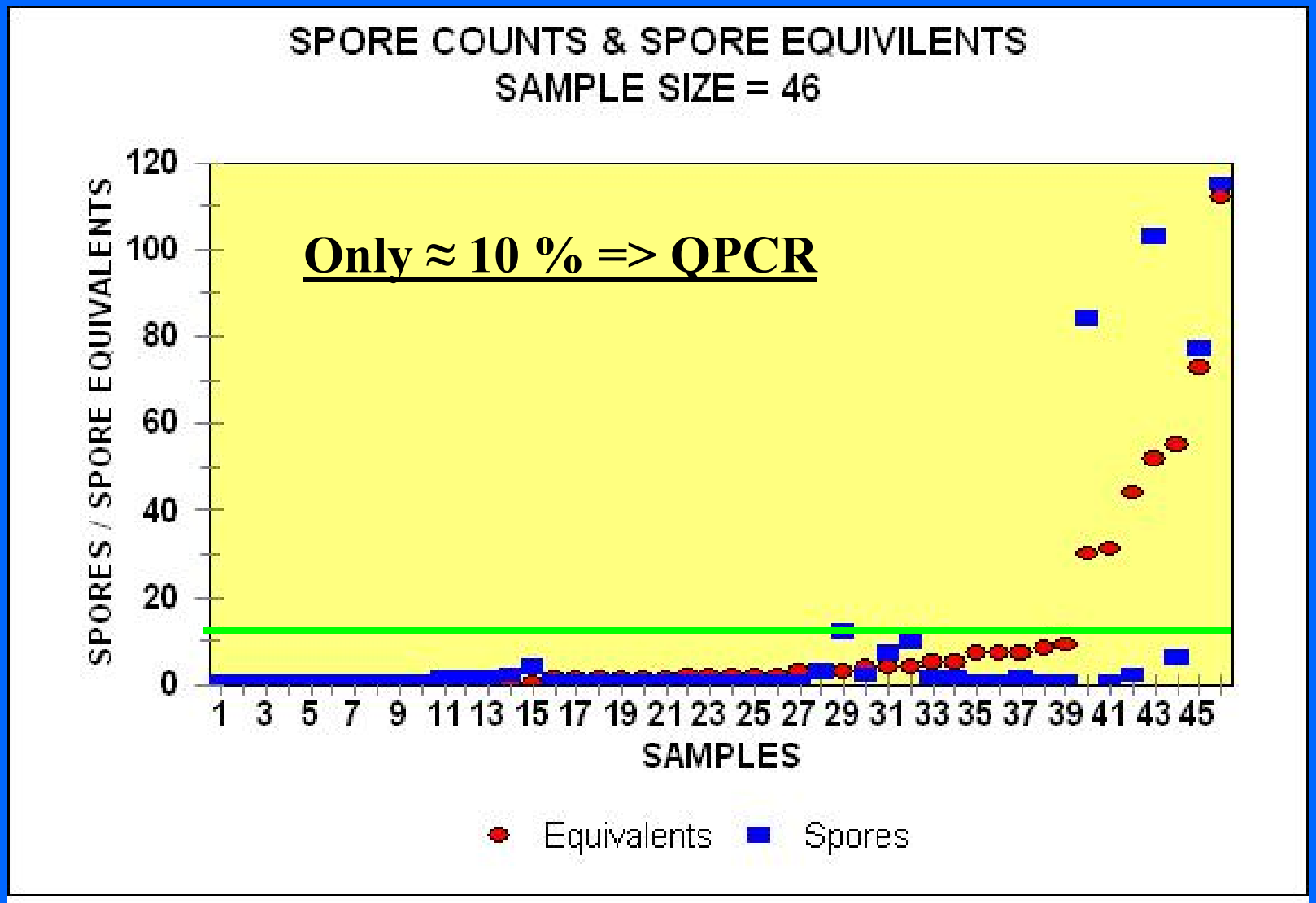
SPORES	SP/M^3	SP EQ*	EQ/M^3
0	0	31	220
0	0	7	74
1	6	7	65
0	0	4	60

*"Common" *Asp* and *Pen*

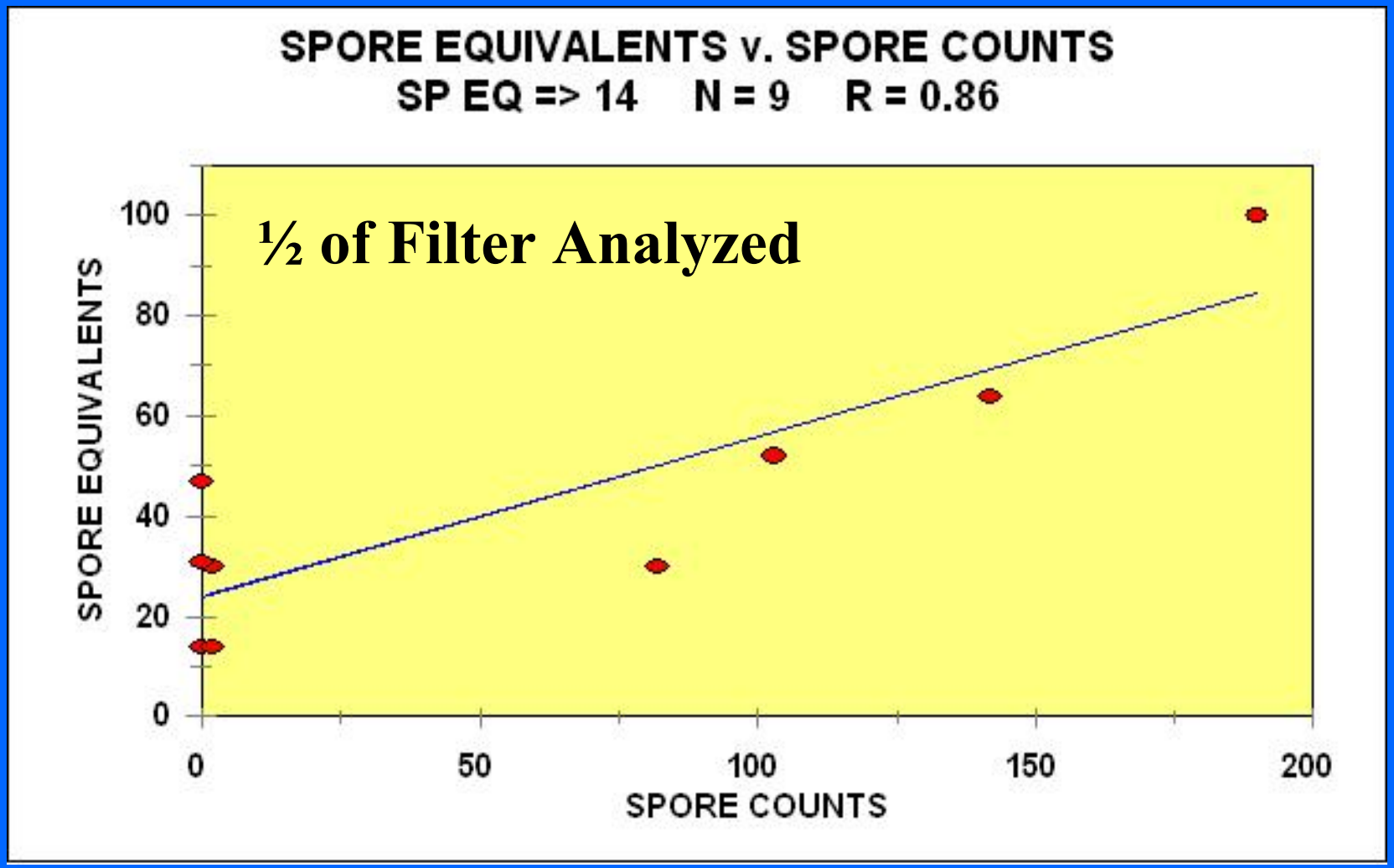
Pass or Fail these 4 OR's ?

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

Rank Order of Sample Results



QPCR: *Asp* & *Pen* on MCE Filters



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/m^3]
      - 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

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"

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/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**

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]

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"]

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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