A New Method for Detecting Low Concentrations of Airborne *Aspergillus* in HEPA-Filtered Hospital Air

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Descriptive Subtitle:

Airborne *Asp/Pen* Spores and *Aspergillus* sp. in Seven Hospitals Using Microscopy and QPCR*

***Quantitative Polymerase Chain Reaction**

Objective

- Develop a rapid, sensitive, cost-effective sampling protocol for detecting *Asp/Pen* spores in highly filtered air with an LOD of 5 spores/m^3 or less

- A simple protocol suitable for:
  - Baseline sampling,
  - Incident response investigations,
  - Post-remediation verification sampling

Assessing Risk Without Cultures?

- **Exposure Assessment**
  - Were *Asp/Pen* spores present? [Microscopy]
  - If no *Asp/Pen* spores, then no *Aspergillus* sp.
  - Negative Exposure Assessment
- **Risk Assessment**
  - If *Asp/Pen* spores were present, then did they include contaminants of concern? [QPCR]
  - *A. fumigatus, A. flavus, A. terreus, A. niger*
- **Risk Management**
  - If yes, then investigate further or remediate

Sampling Protocol

- Collect duplicate TWA* fungal spore samples using the Bi-Air Filter Cassette
- Analyze 100 % of one sample by microscopy at 600X magnification
- Analyze duplicate sample by QPCR only if *Asp/Pen* spores were detected in the first sample and a risk assessment was needed

Time-Weighted Average

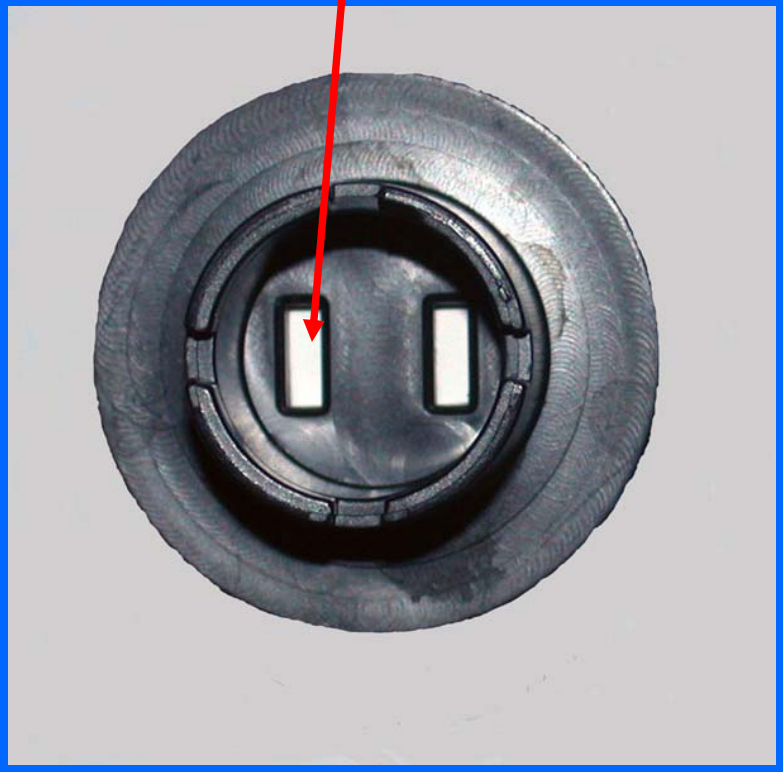
Bi-Air Filter Cassette: 25 mm

Flow Rate = 0.5 – 3 lpm

Four air inlets; vary width for constant capture velocity

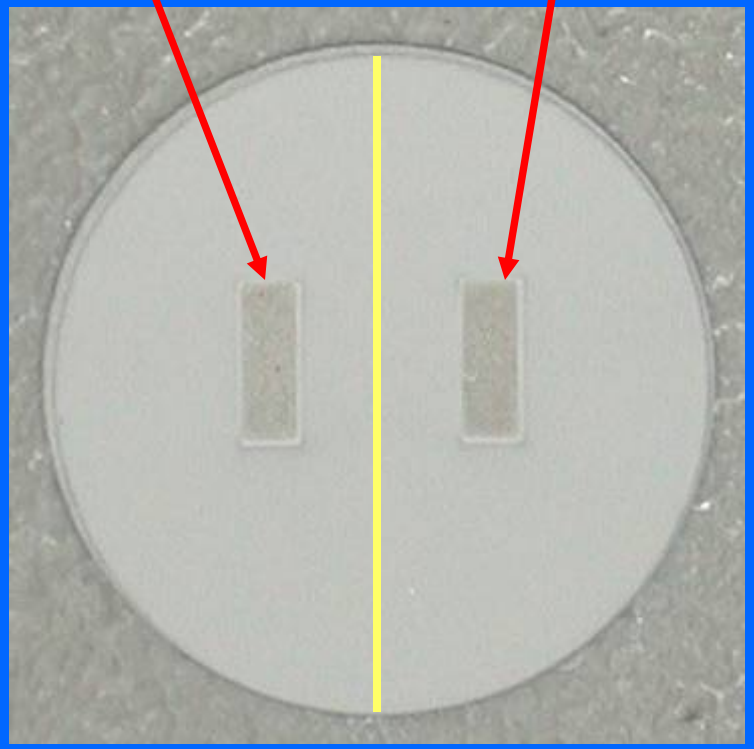
Duplicate TWA Samples on an MCE Filter

9.35 mm^2



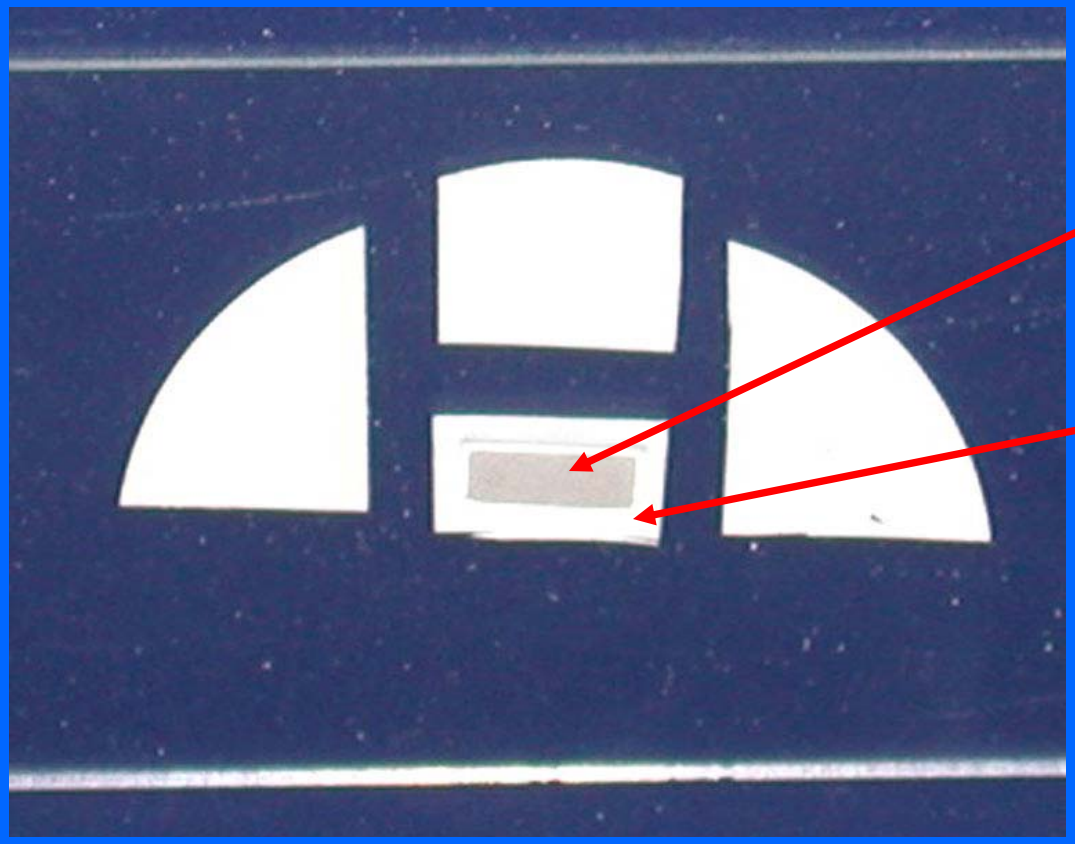
Concentration Factor = 20.6

Microscopy Archive*



*QPCR or Culture

One Bi-Air Sample Trace



- Sample trace = 2 % of total filter area
- Analyze 4 % of the total filter area by QPCR

25 mm MCE Filters and QPCR Analysis

Background:
Sp-Eq / Filter

0
0
3
27
37
156

6 blank MCE filters

Range = 0 - 156

Avg = 37 Sp-Eq/Filter

Unacceptable

Background:
Sp-Eq / Sample Trace

0
0
0.1
1.1
1.5
6.2

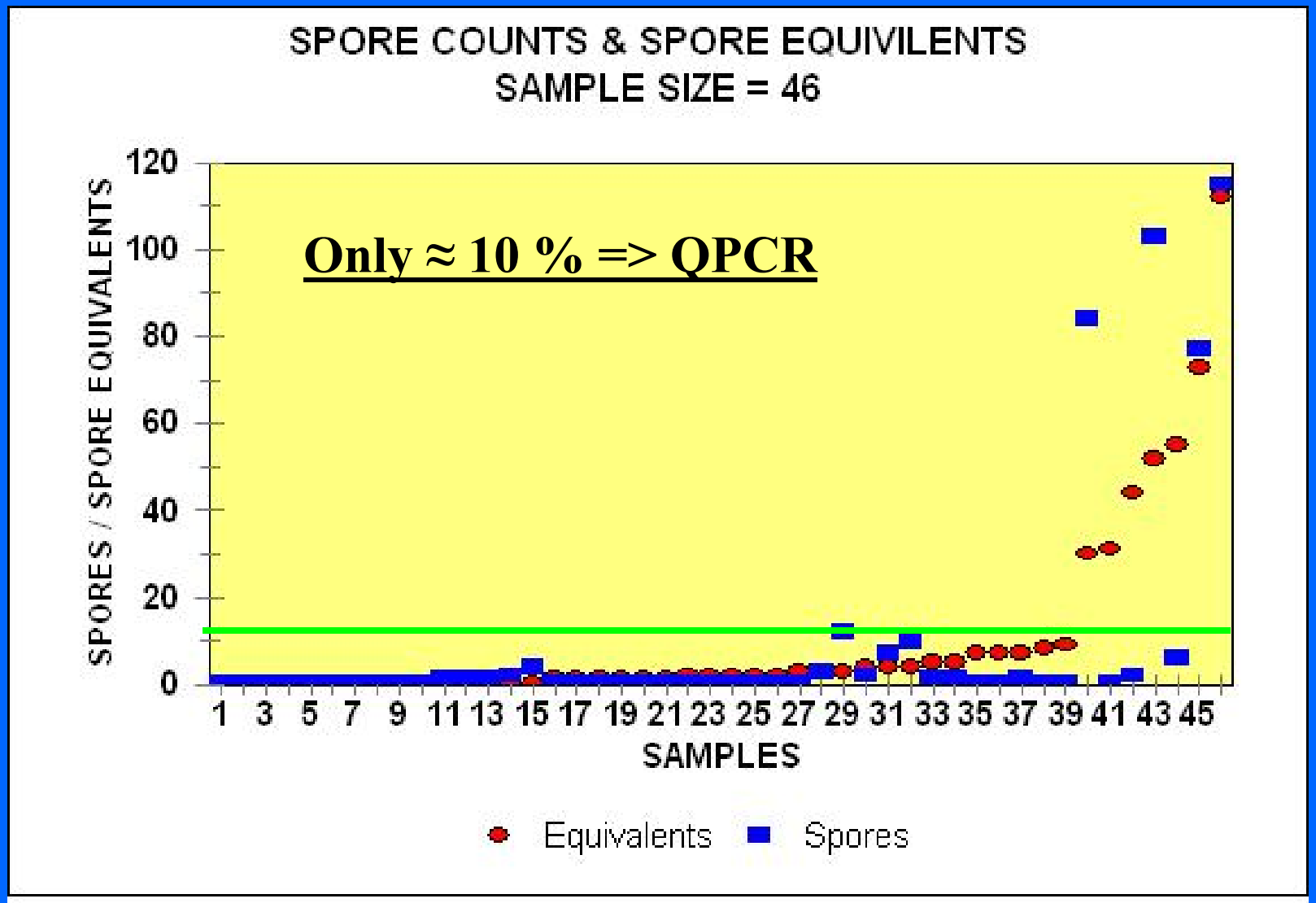
6 blank Bi-Air Traces

Range = 0 – 6.2

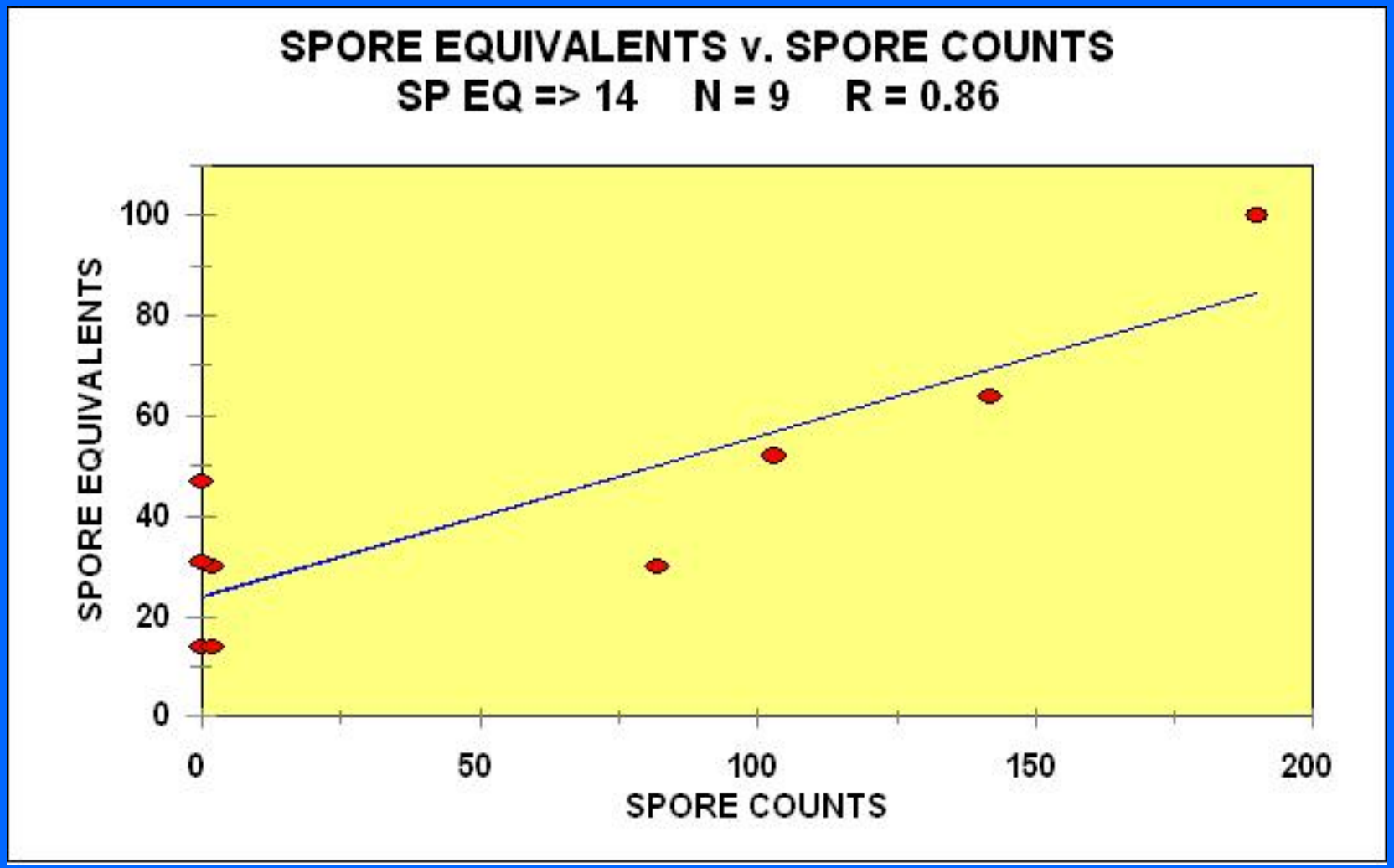
Avg = 1.4 Sp-Eq/Filter

Usable

Rank Order of Sample Results



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



Asp/Pen Spores by Microscopy: Spores/m^3

LOCATION	SAMPLES	GM	AVG	95th %-tile
Op Rm	20	0.8	1.7	3.8
ICU	62	1.1	6.9	12.8
Post Op	10	2.8	17.4	117.4

7 Hospitals

Asp and Pen by QPCR: Sp-Eq/m^3

LOCATION	SAMPLES	GM	AVG	95th %-tile
Op Rm	11	1.7	4.5	14.3
ICU	10	6.5	23.5	140
Post Op	4	225	635	4,880

7 Hospitals

Problem Detection: Sensitivity and Sampling Time

- **Problem Operating Room**
  - Surgeons refusing to use an OR
  - 10-min Air-O-Cell sample
    - "No problem" – place back in service
  - 3-hour Bi-Air sample
    - 4 *Asp/Pen* spores
      - Detected one *Asp/Pen* spore / 45 minutes – "rare event"
    - One *Stachybotrys* spore
  - Recommendation: inspection by facilities
  - Result: Two walls remediated

ICU Clearance by Microscopy

- 13 locations sampled
- 7-hour BA samples at 3 lpm
- Volume = 1,260 liter
- LOD = 0.8 spores/m^3
- Microscope on-site
- *Asp/Pen* spores, no QPCR
- 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

- **Collect TWA Samples v. Short-term Grab Samples**
  - Lower LOD – less than 1 spore/m^3
  - Fewer false negatives - more confidence in decisions
- **Cost Efficient Exposure Assessment**
  - Same LOD = One BA v. 9 AOC or 9 N6 samples
  - Minimizes number of expensive QPCR samples
- **Rapid Risk Assessment**
  - Two days for QPCR v. 10-12 days for culturing
- **Utility for Incident Investigations**
  - Culturing - Number of spores per cfu is undefined
  - Simple protocol for Baseline, Incident Investigation, and PRV

Characterizing Hospital Environments