

US EPA ARCHIVE DOCUMENT

 **How Sampling Methods Influence Overall Exposure Assessment**

Microbial Risk Assessment  
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 **Outline**

- Purpose of Sampling
- Sampling Methods
  - Culturable
  - Non-Culturable
- Pros and Cons
- Summary



2 of 23

 **Purpose of Sampling**

- Exposure Assessment
  - Risk
- Identify microorganism (strain, family or class)
- Quantitative or qualitative
- Culturable and Non-culturable
- Size distribution
- Endotoxin / mycotoxin

3 of 23

 **Risk-based Environmental Concentration**

$Risk = Intake \times Toxicity$

Media Concentration → Exposure

4 of 23

 **Sampling Methods**

- Sampling characteristics
  - Sampling efficiencies
    - Inlet
    - media collection
    - recovery
  - Sampling flow rate
  - Sampling media
  - Analysis



5 of 23

 **Sampling Methods**

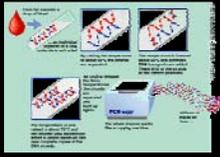
**Culturable**

- ID & enumerate organism
- Determination if alive
- Analysis: Plate culture, enumeration, stain, biochemical, etc.

**Non-Culturable Sample**

- ID & enumerate organism
- Analysis: Microscopy, Immunoassay, PCR





6 of 23

## Air Sampling - Summary

Equipment *	Media	Culture
Impactors	Agar Plates	Y
	Collector Plates (Filter, Glass, Tape, SS)	N
Impinger	Liquid Media	Y
Filter Cassette	Gel Filters	Y
	Filters (MCE, PVC, PTFE)	N, but...

\* Sample pump required but not listed

7 of 23

## Examples of Impactors

8 of 23

## Impactor *sampling characteristics*

- Multi-stage or single stage impactors
- Size distribution down to submicro

Characteristics	Culture	Non-Culture
Media	Agar (selective)	glass slide, stainless steel, tape, filters
Sample Efficiency (Collection)	Very good	Fair (sample loss due to particle bounce)
Sample Time	1-10 minutes*	1-10 minutes*
Flow Rate	28.3 LPM	10-100 LPM

\*Longer sampling times with rotating media type – hr to days

9 of 23

## Impactor with Agar

<p><b>Pros</b></p> <ul style="list-style-type: none"> <li>Culturable sampler</li> <li>Nutrient media is selective</li> <li>Size distribution data</li> <li>Very good sampling efficiency</li> <li>Good for low concentrations</li> <li>Identify species</li> <li>large reference database</li> <li>no special equipment</li> </ul>	<p><b>Cons</b></p> <ul style="list-style-type: none"> <li>Slow results</li> <li>Underestimate total conc. (culturable only)</li> <li>Easily overloaded high conc</li> <li>no dilution</li> <li>Short sampling time</li> <li>labor intensive</li> <li>Nutrient media is selective</li> <li>Complicated data interpretation</li> </ul>
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10 of 23

## Impactor- non-Cultureable

<p><b>Pros</b></p> <ul style="list-style-type: none"> <li>Non-culturable sampler (microscopy)</li> <li>Size distribution data</li> <li>Easy to use</li> <li>Fast and affordable</li> <li>total microorganism loading</li> </ul>	<p><b>Cons</b></p> <ul style="list-style-type: none"> <li>Short sampling time - can improve with rotating drum</li> <li>Sample loss due to particle bounce</li> <li>Limited identification between some species</li> <li>Special expertise needed</li> <li>Nonspecific - culturable and non-culturable</li> </ul>
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11 of 23

## Example of Impingers

12 of 23

### Impinger *sampling characteristics*

Characteristics	Culture
Media	Liquid – sterile DI water, PBS, nutrient broth, peptone water
Sample Efficiency (Collection)	Fair/Good 90-100% at 1 um (swirl sampler) <u>NOTE: decreases for spores</u>
Sample Time	30 min or less
Flow Rate	12.5 LPM

13 of 23

### Impinger

- Pros
  - Culturable sampler
  - Identify species
  - Multiple analysis on liquid media (culturable and non-culturable)
  - Low to high concentrations (dilutions)
- Cons
  - Sample loss - particle/liquid interaction and media evap,
  - Short sampling time
  - labor intensive
  - Slow results
  - Underestimate total concentration (culturable only)
  - complicated data interpretation

14 of 23

### Examples of Filter Samplers

15 of 23

### Filter *sampling characteristics*

Characteristics	Culture	Non-Culture
Media	Gelatin Filter	Cellulose ester membrane, polycarbonate, teflon, polyvinyl chloride
Efficiency (Collection)	Good Collection - > 90%	Good Collection- > 90% with 3 um or small filter pore size Recovery- > 90% for spores – less for others
Sample Time	30 - 45 minutes maximum	Minutes to hours
Flow Rate	1-5 LPM	1-5 LPM

16 of 23

### Filter Sampling Summary

- Pros
  - Non-culturable and culturable sampler
  - Low to high concentrations
  - Longer sampling periods (hours)
  - Good sample collection efficiencies
  - Recovery liquid – multiple analysis
  - Fast and easy to use
  - Measure total microorganism loading
  - Use as a personal sampler
- Cons
  - Sample loss (recovery efficiency)
  - Culturable sampler – desiccate and kill microorganism
  - Debris can interfere with identification (microscopy)

17 of 23

### Surface Sampling

- Swabs
- Wipes
- Vacuum with filter sock

18 of 23



## Surface Sampling

- Culturable and non-culturable sampling
- Swabs and Wipes– non-porous surface
  - Non-cotton material – rayon, polyester, etc.
  - Wetted - distilled water, PBS, and/or Tween
  - 100 cm<sup>2</sup> for swabs / 930 cm<sup>2</sup> for wipes
- Vacuum with filter sock – porous surface
  - large surface area (up to 3600 cm<sup>2</sup>)

19 of 23



## Surface Sampling

- **Pros**
  - Fast, easy and available
  - Wipes – large sampling area / composite
  - Vacuum – large sampling area / porous surface
  - Recovery liquid – multiple analysis
- **Con**
  - Main problem – poor sampling efficiency (< 40%)
  - Swabs – small sampling area
  - Vacuum – stress organism (culture)

20 of 23



## Conclusion

- Method selection influences exposure assessment
  - Sampling efficiencies
  - Collection media
- No “one method fits all”



21 of 23



## Conclusion

- Selection depends on
  - purpose of sampling
  - Microorganism
  - Sampling characteristics

22 of 23



## Questions?

23 of 23