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Don’t Get Spooked by Analytical Testing

Erin Bouttenot, Laboratory Director

"...study design is often the single most important element of an investigation. Even the most careful and sophisticated sample collection and data analysis will not salvage a poor study design."

- ACGIH *Bioaerosols: Assessment and Control* (1999)

Foundational Steps

Foundational Steps

A. Identify client goals.

B. Health assessment.

C. Building assessment.

Foundational Steps

A. Occupant complaints and potential causes.

B. Possible sources of mold.

C. The building environment.

Foundational Steps

A. Create and perform an observation/sampling plan.

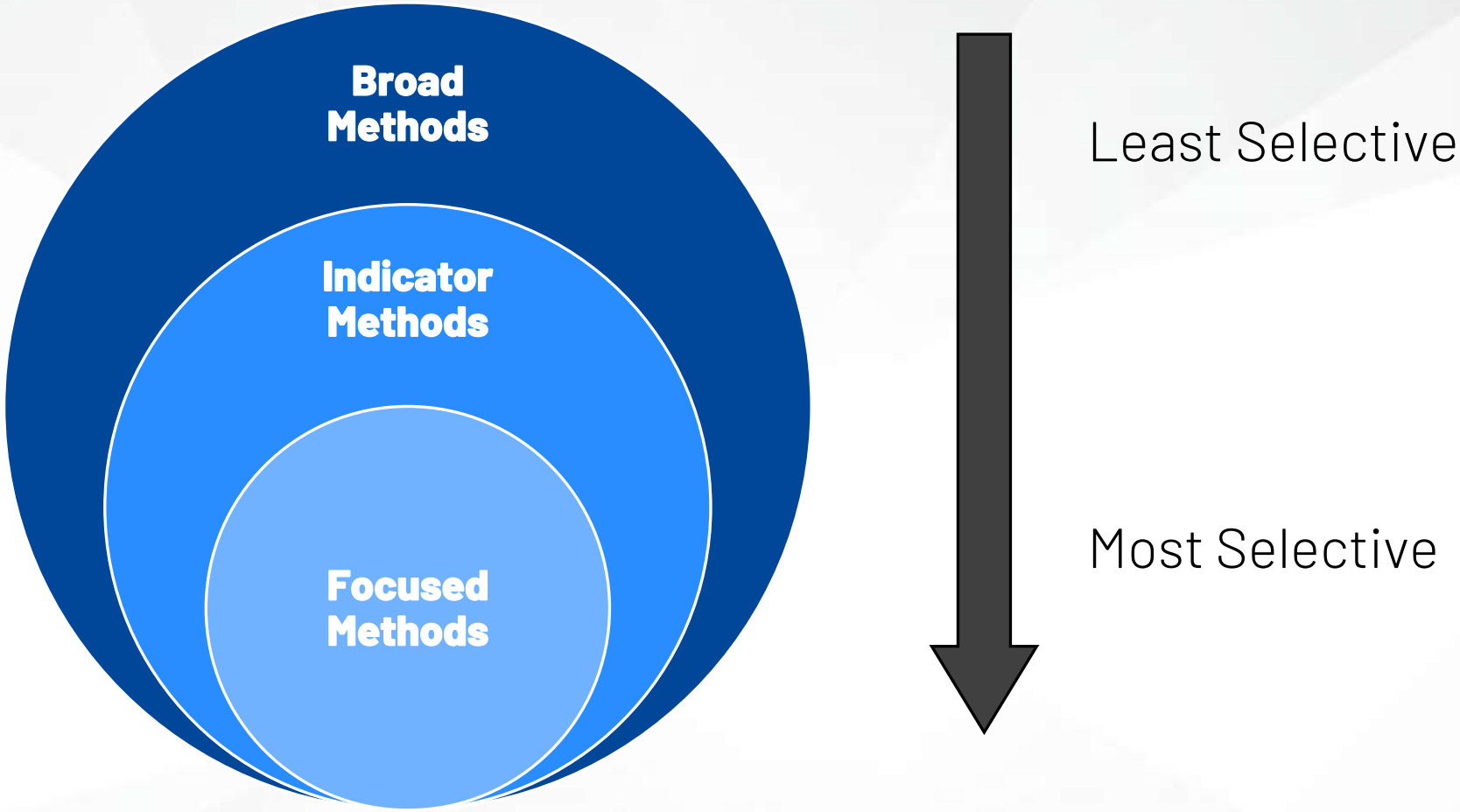
B. Interpret all data collectively.

Foundational Steps

A. Repair & replace.

B. Prevention.

Choosing an Analytical Method



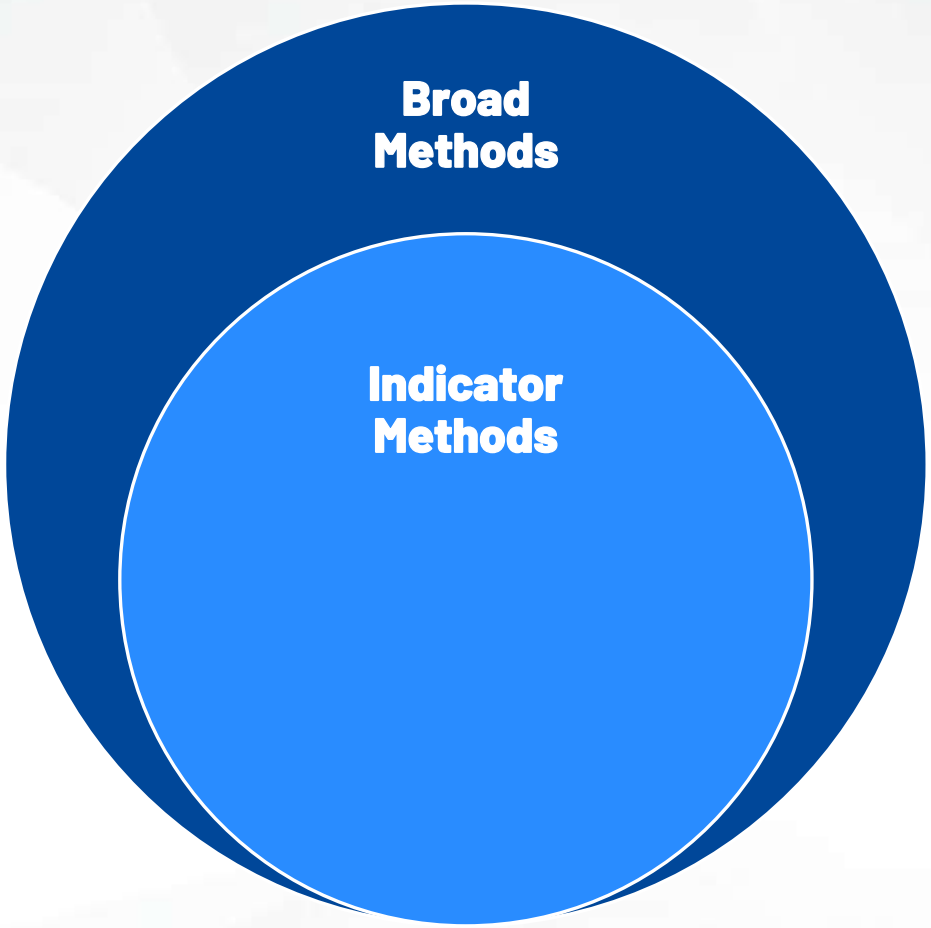
Broad Analytical Methods

Direct Microscopy

Molecular Groups

Chemical Groups

Indicator Analytical Methods

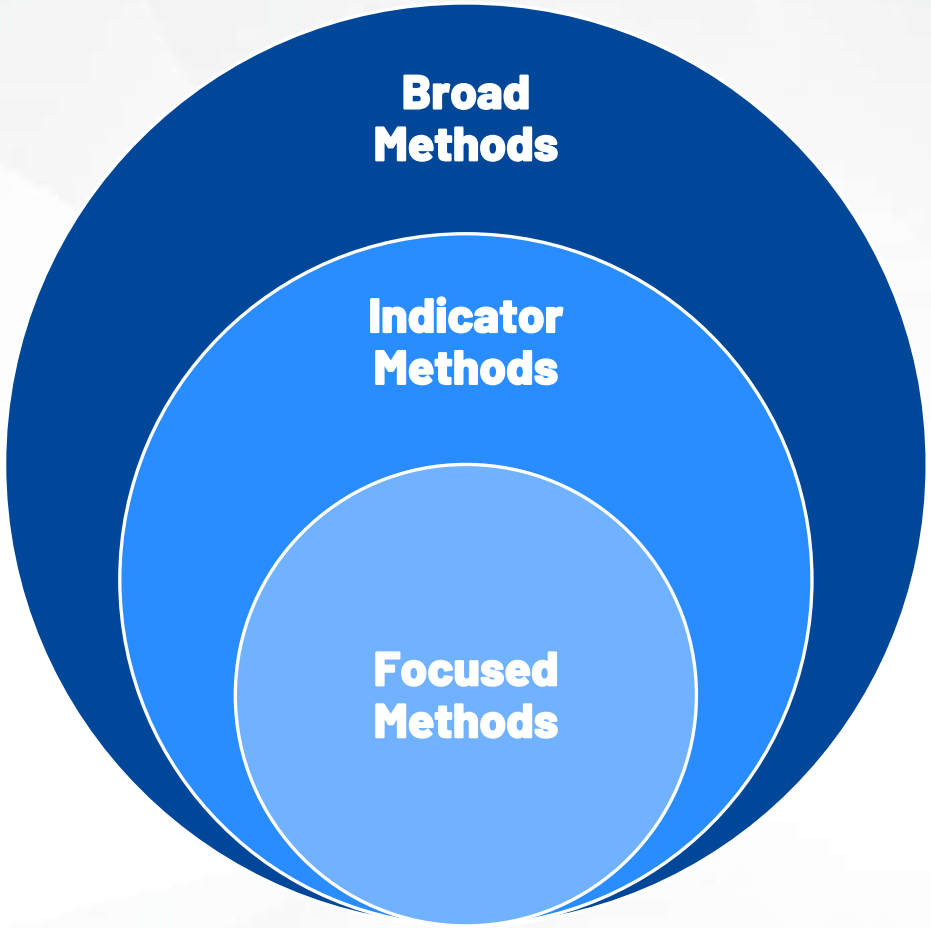


Total Coliforms or *Escherichia coli*

ATP, Glucan, or Ergosterol

Bacteria, Amoebae, or Ciliates

Focused Analytical Methods



Culture Methods

PCR

Bioassays

Culture Analysis

- Single or multiple cells can produce a CFU (bacteria/fungi) or PFU (viruses), but only cells that are culturable.

- Only cells that are living and can survive and replicate in the growth conditions provided will be detected.

Culture Analysis

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Air Sampling

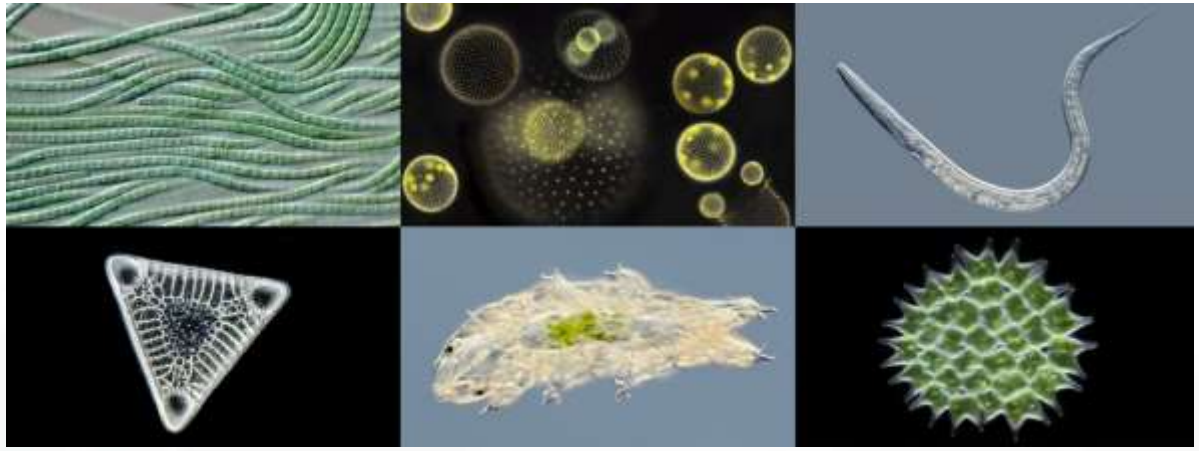
- Air Impaction
- Liquid Impingement
- Filtration

Surface Sampling

- Wiping/swabbing
- Cutting
- Vacuuming
- Contact

Microscopical Analysis

- Detection of both culturable and nonculturable bioaerosol agents.
- Many different types of microscopes, filters, and stains.
- Identify what is best suited for the target agent based on the agent's cell size, color, and complexity.



Microscopical Analysis

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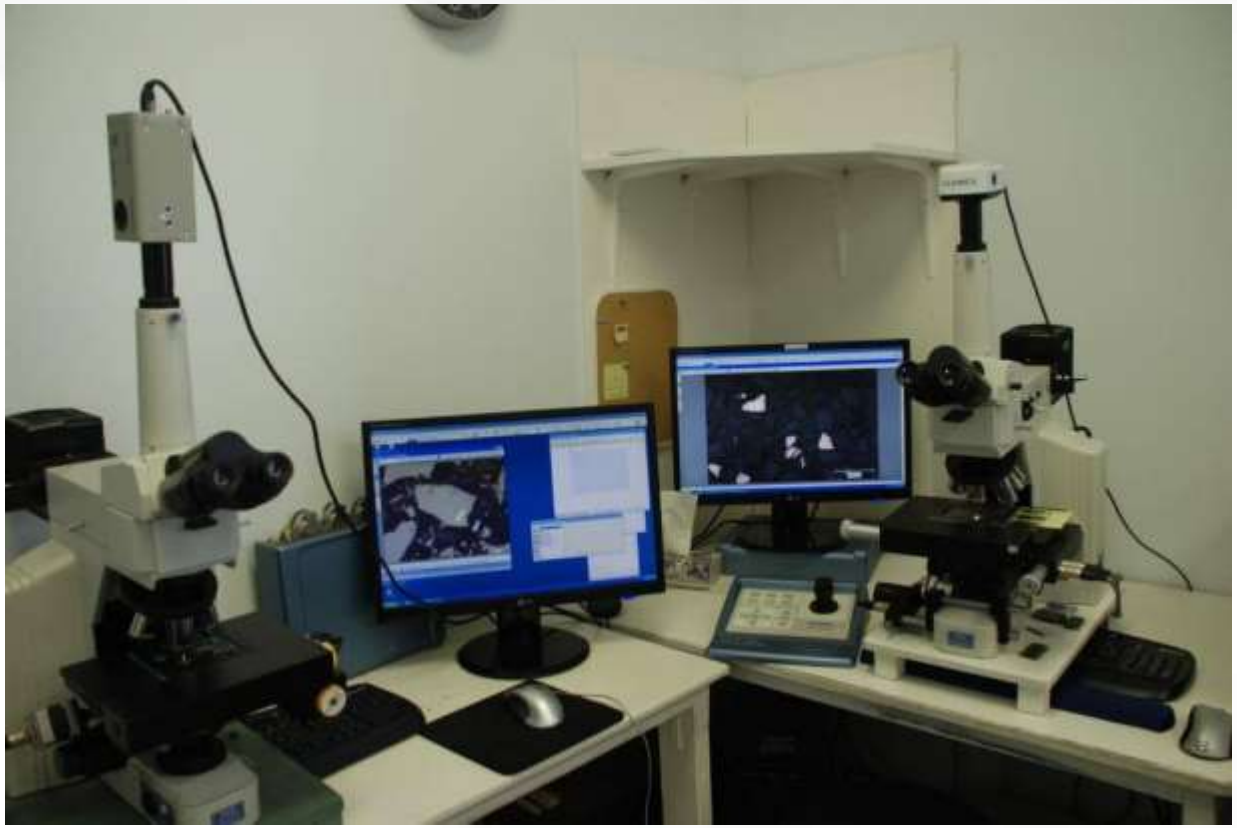
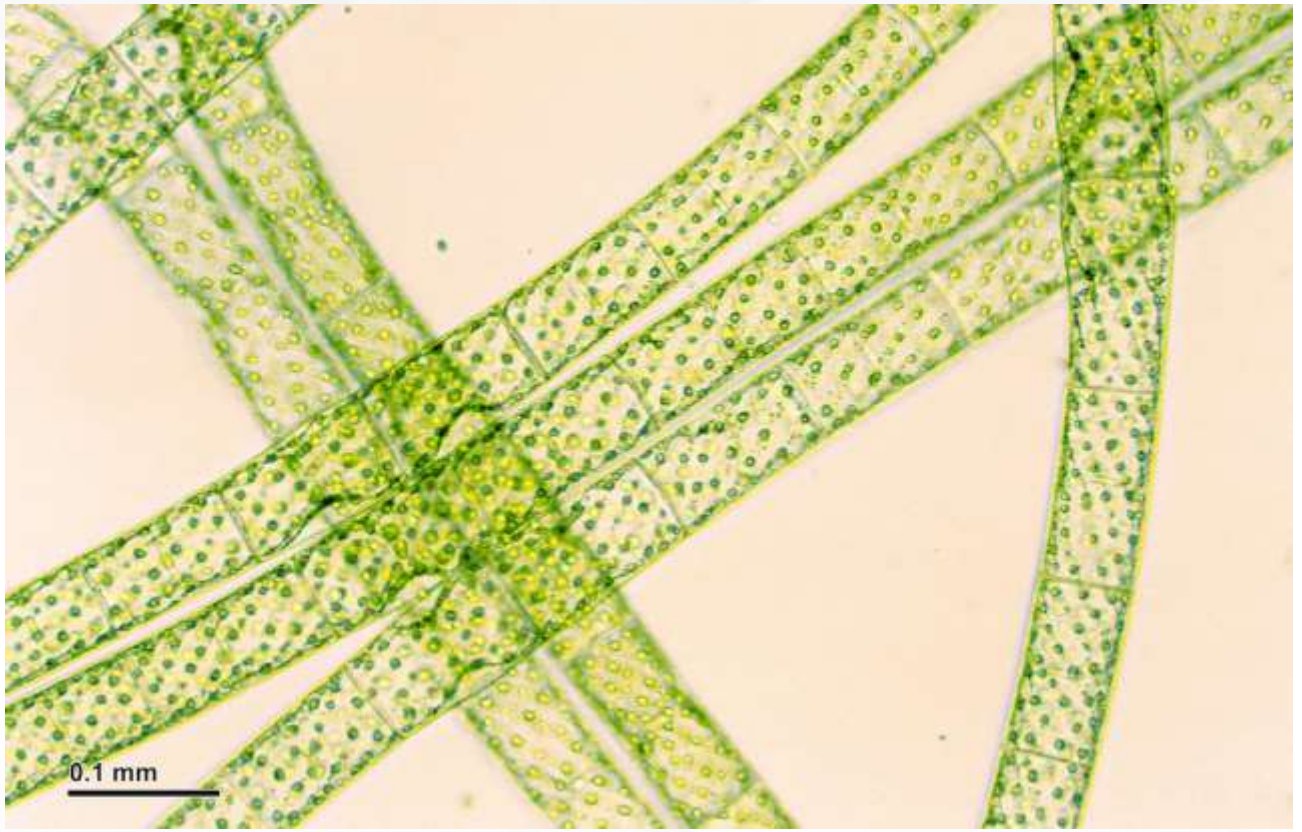
– Light Microscopy





Microscopical Analysis

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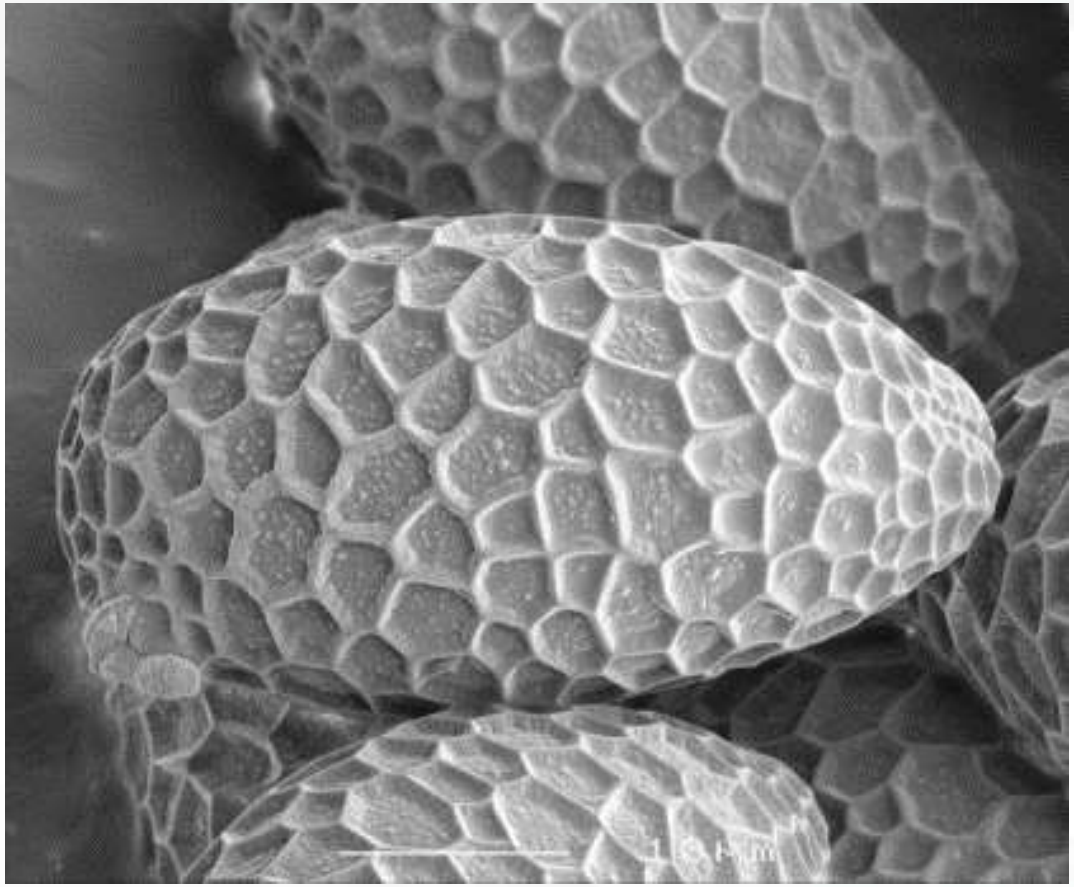
– Scanning Electron Microscopy



Microscopical Analysis

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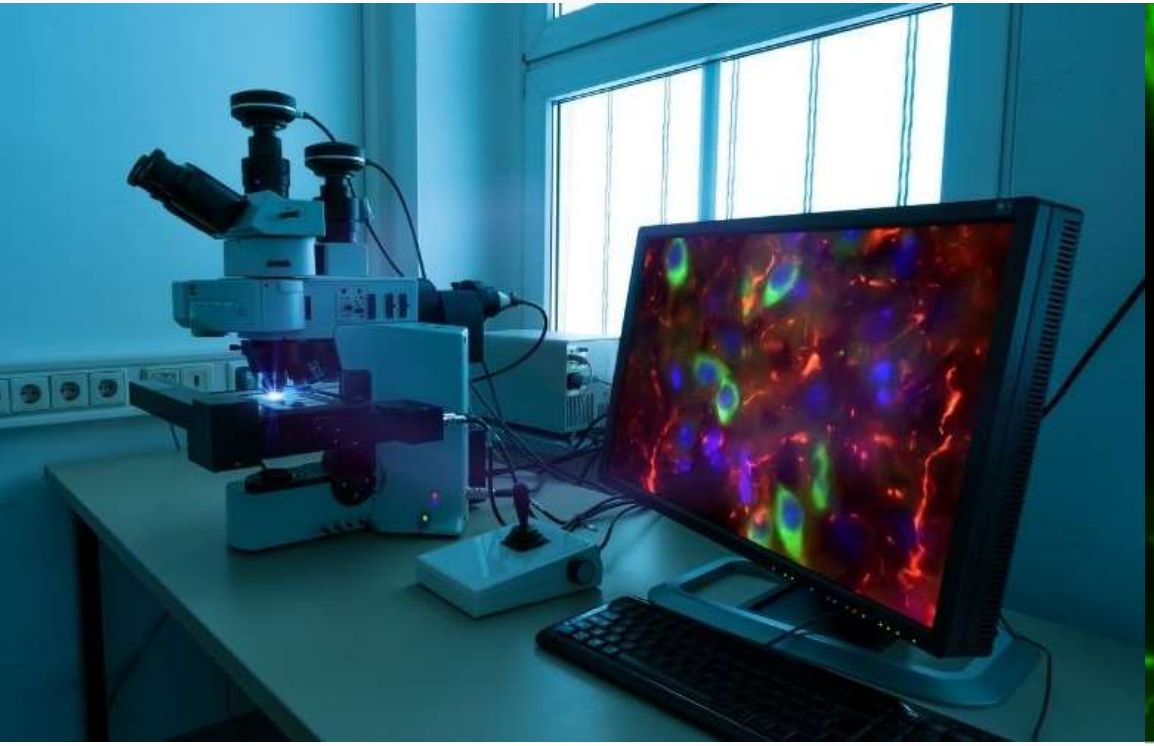
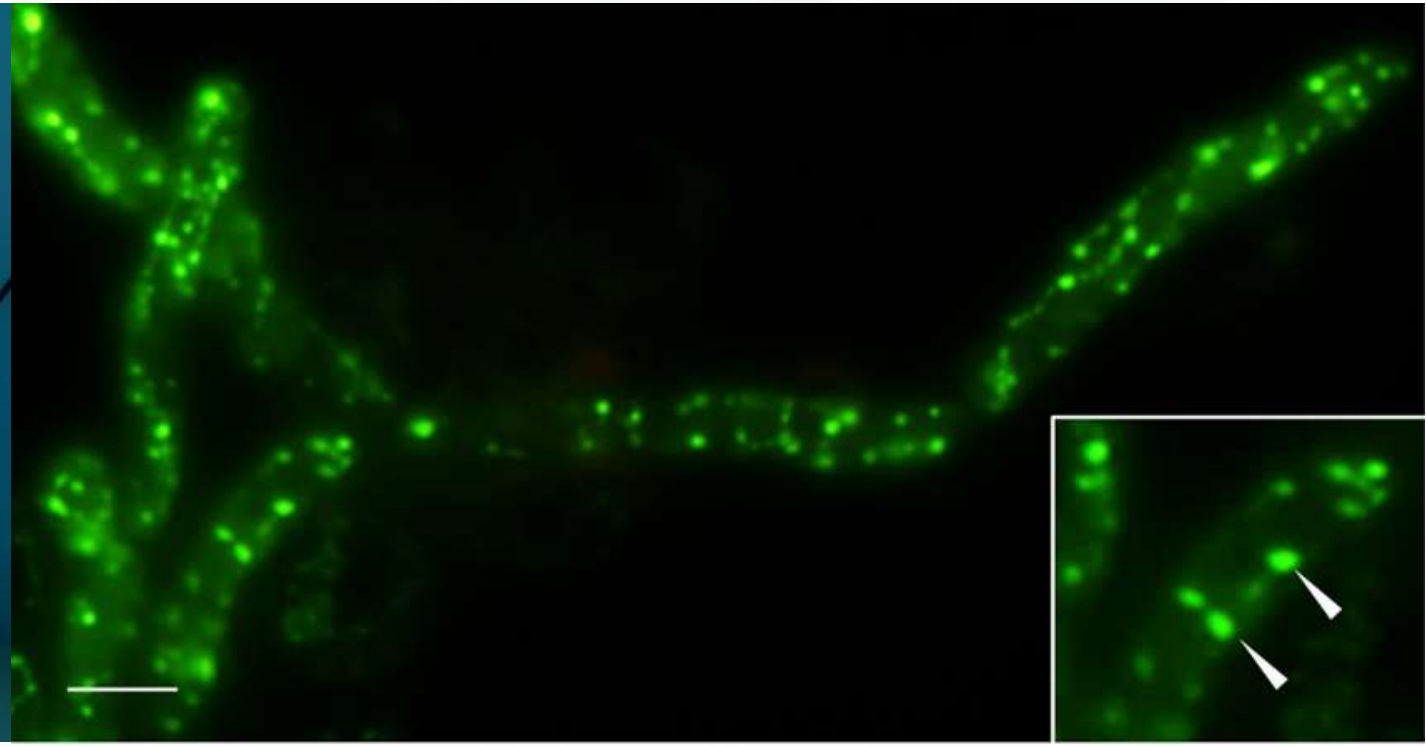
– Fluorescent Microscopy





Bioassay Analysis

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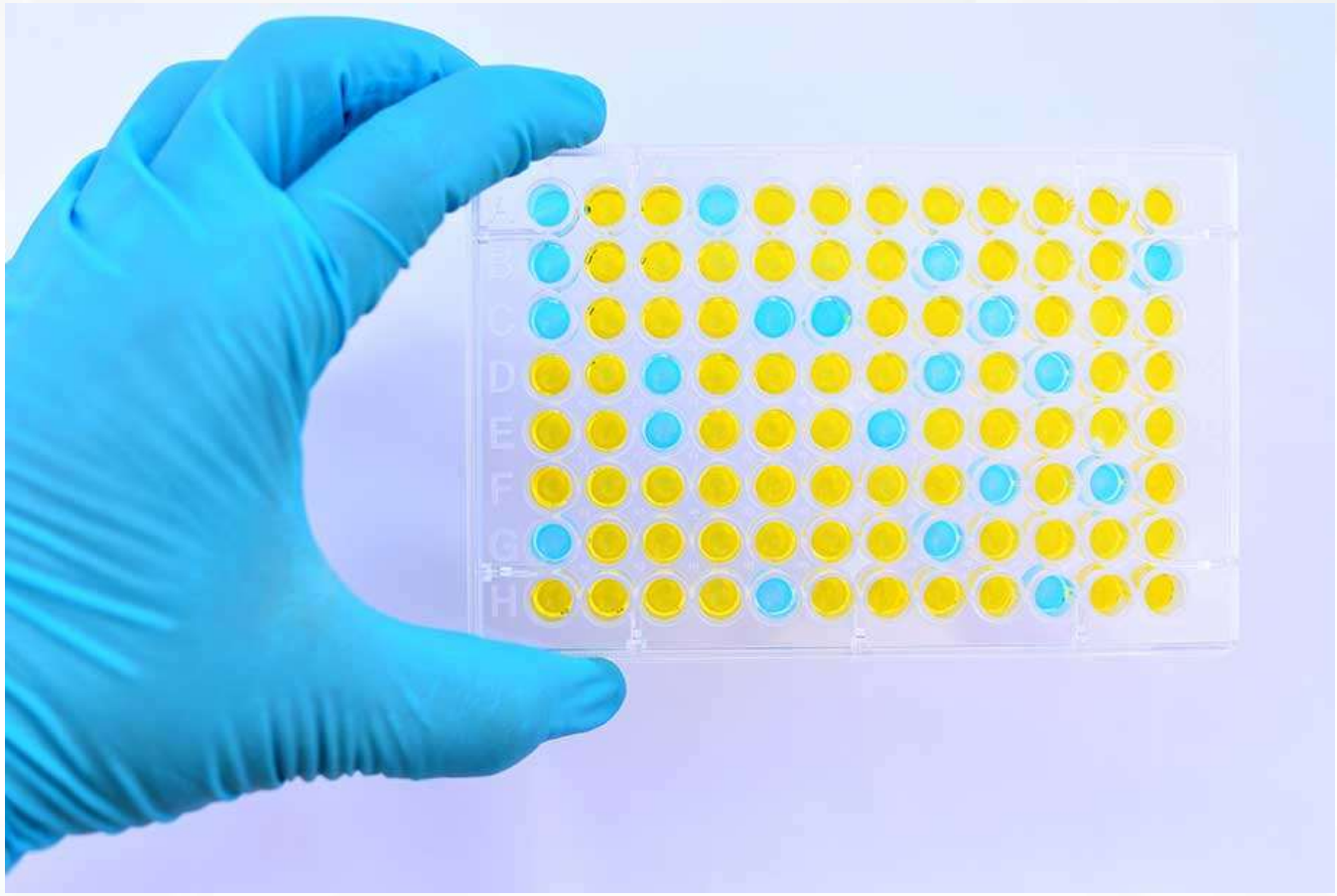
– Immunoassays



Bioassay Analysis

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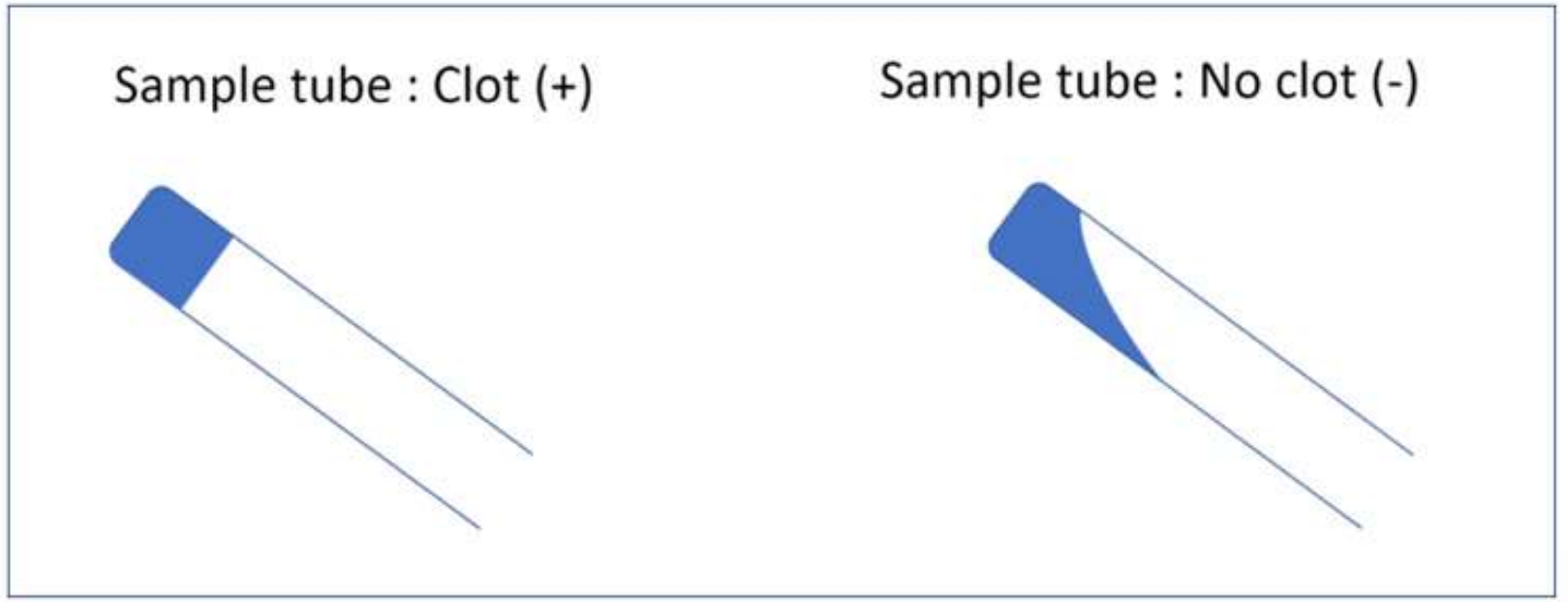
– Limulus Amoebocyte Lysate (LAL) Assay



Bioassay Analysis

– ATP Bioluminescence Assays

Step 1

Use special swab to sample surface

Step 2

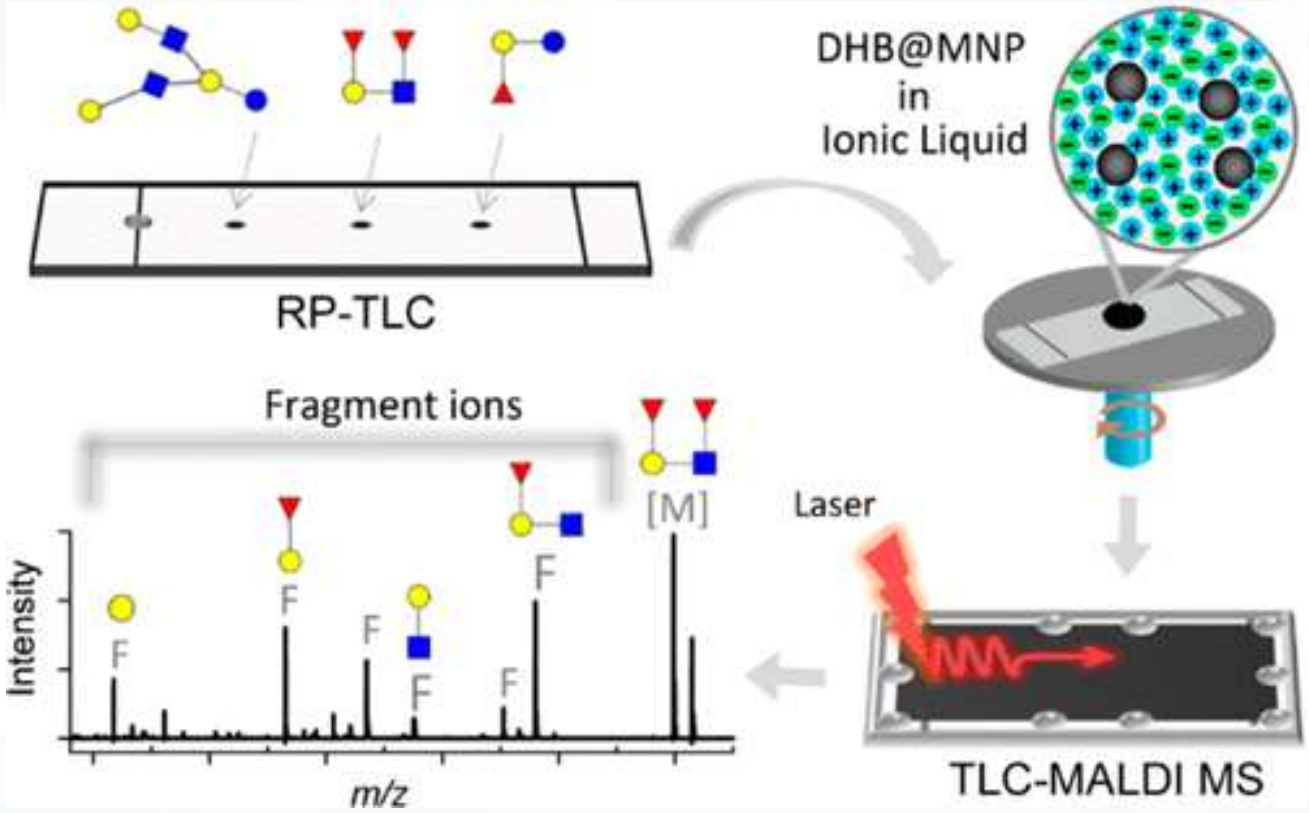
Place swab in reaction tube

Step 3

Place tube in luminometer Results: Relative Light Units

Chemical Assay Analysis

– HPLC, GC-MS, GLC, TLC, MALDI-TOF



PCR Analysis

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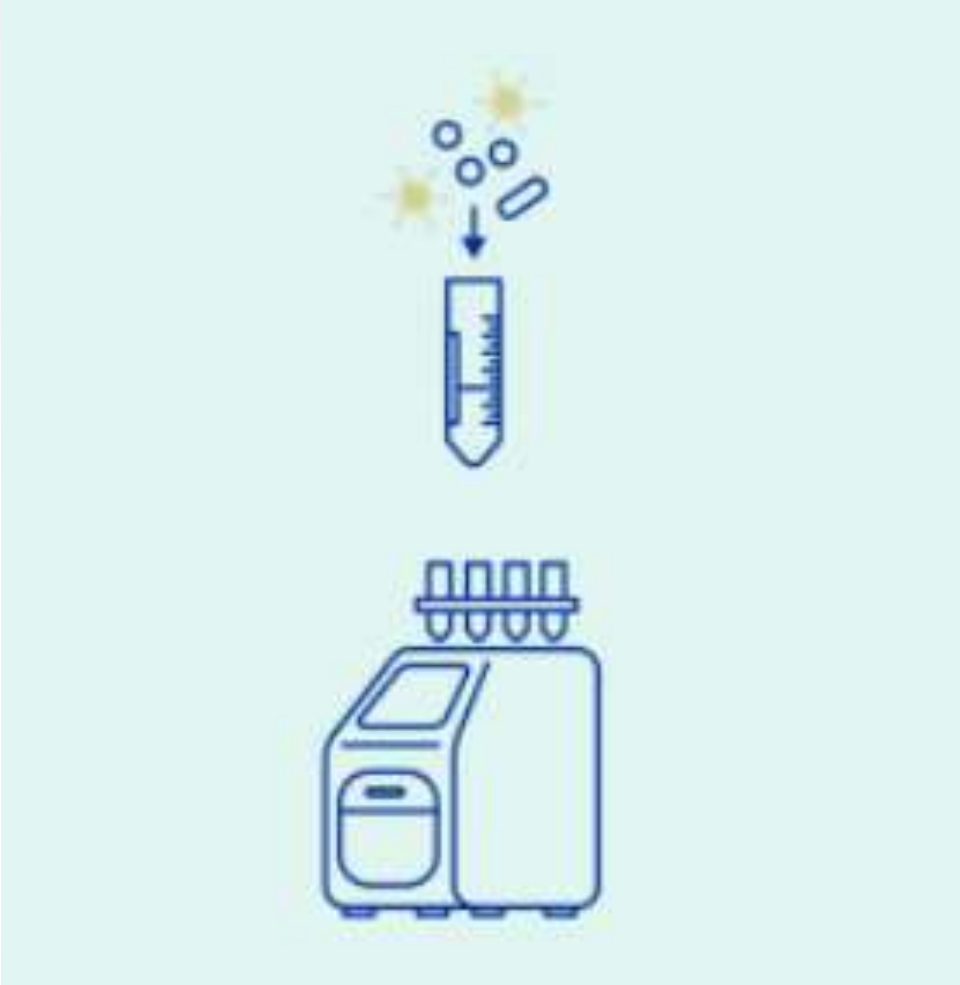
Polymerase Chain Reaction (PCR)

- Nested PCR
- Multiplex PCR
- Broad-range PCR
- Quantitative-competitive PCR
- Genomic fingerprinting PCR
- Reverse transcriptase PCR (RT-PCR)
- Real-time PCR (qPCR).



Not all tools are the same

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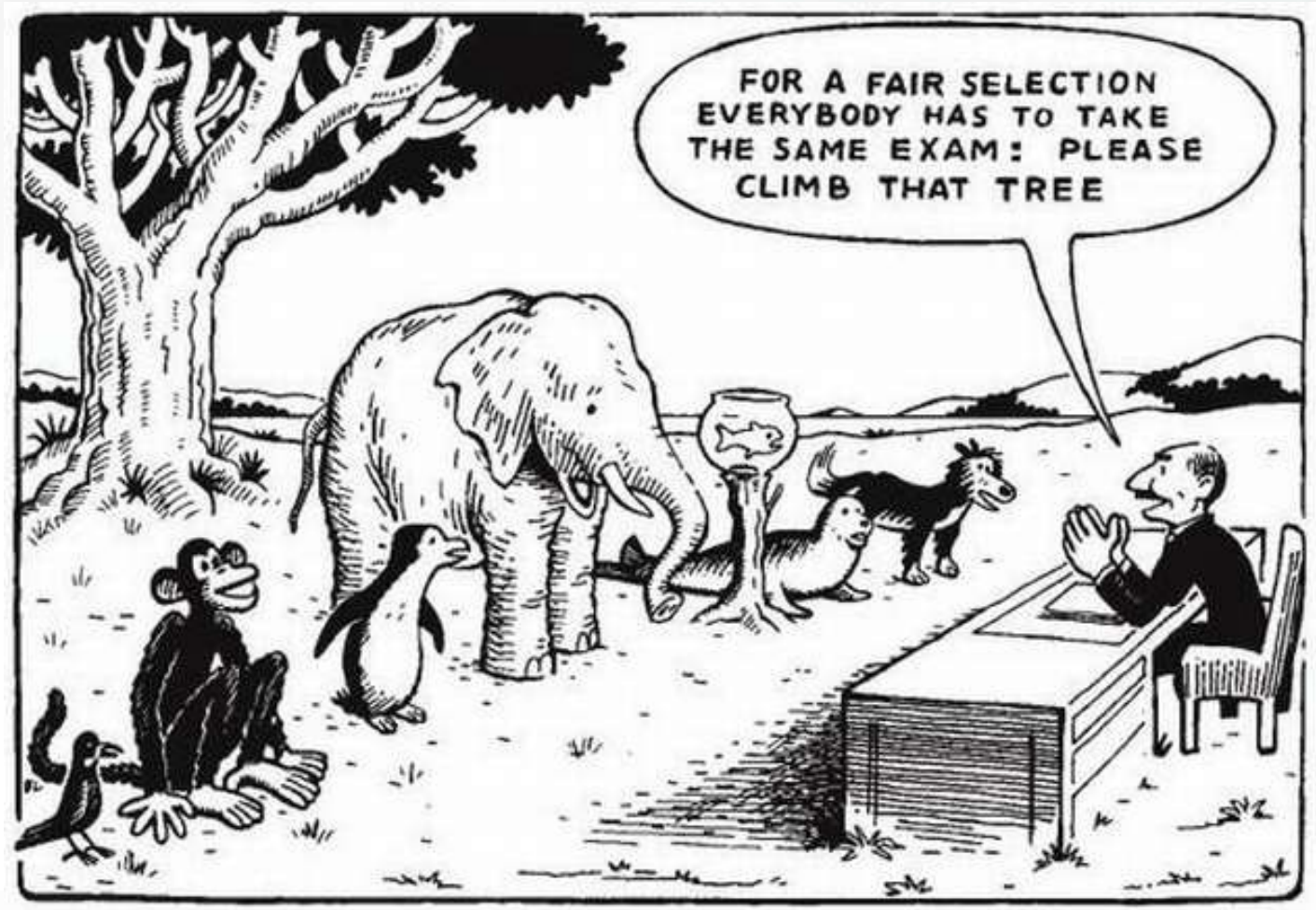
Understanding Bias



Know Before You Buy

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Qualifications of Laboratories and Laboratory Personnel

- ISO/IEC 17025
- AIHA Laboratory Accreditation Programs, LLC
- ASTM International
- Formalized training programs

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Laboratory Proficiency

- Proficiency Analytical Testing (PAT) Program
- Environnemental Legionella Isolation Technique Evaluation (ELITE) program
- State proficiency programs

Know Before You Buy

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Laboratory Quality Control

- Data defensibility
- Standardized procedures whenever possible
- QC protocol such as references and comparison tests
- Protection of samples from improper handling and storage
- Documentation of all sample-related information

Pre-Test Conditions

1. No unusual cleaning.
2. Close windows at least 7 hours before sampling.
3. Don’t vacuum at least 24 hours before sampling.

Outdoor Sample(s)

Purpose = identify possible contaminates

1. Ideally 3 feet above ground, away from possible outliers, and upwind from property.

2. Should be taken wherever outdoor air may enter the building.

3. Note outdoor environment in proximity of house and samples.

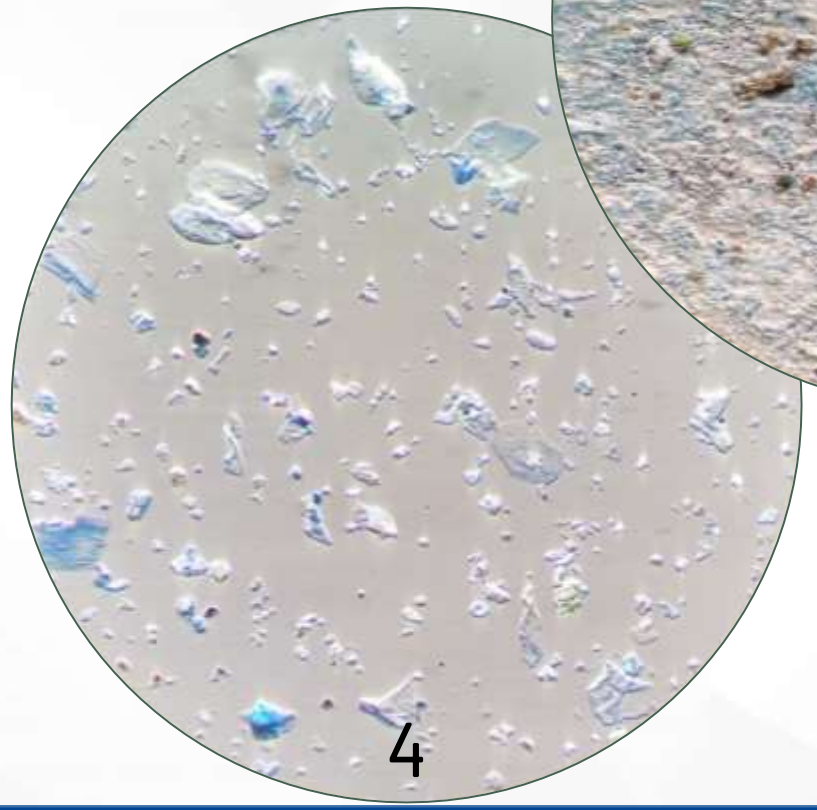
Indoor Samples

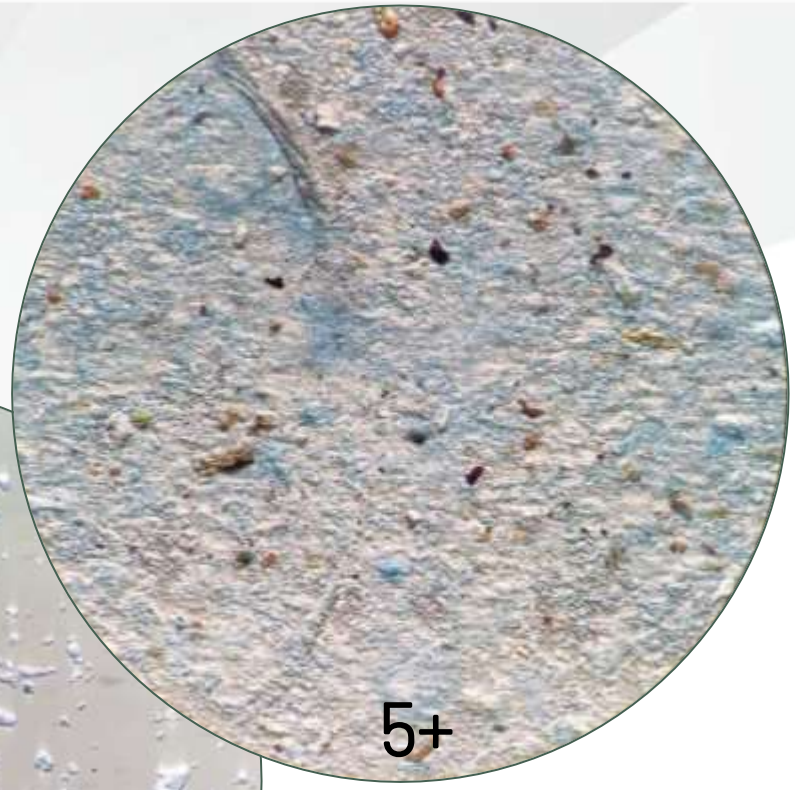
Purpose = assess typical and worst-case exposure

1. Ideally taken at average breathing zone height.
2. Center of room/area and away from possible air pathways.
3. Close off the area being sampled as much as possible.
4. Exhaust of pump directed away from suspect growth.
5. At least two samples from each area simultaneously or duplicated over time.

Sample Volume

- Reflected in Background Debris Rating
- Adjust to the cassette brand
- Adjust to the environment





Sampling Intervals

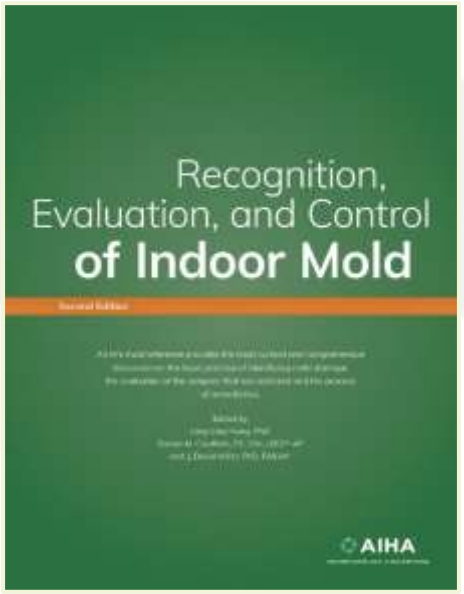
Cassette Brand	Dust Free	Occupied	Excessive Dust	Outdoor
	5-8	3-5	1-3	1-10
	10	5	0.5	10-60

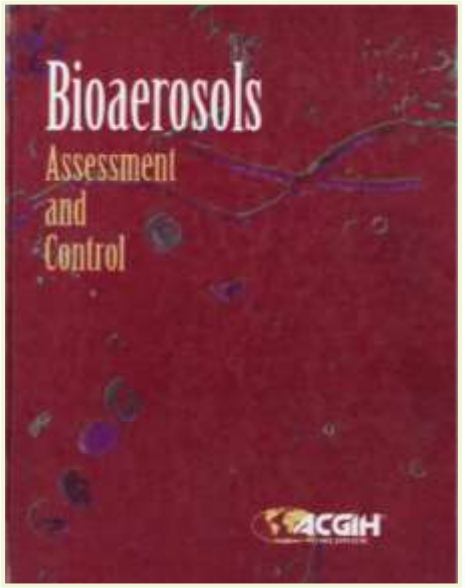
Flow Rate = 15 LPM

Recommended to take at least all indoor samples at the same sampling interval.

Easy Quality Control

1. Check expiration date of supplies.
2. Prevent temperature extremes.
3. Check pump flow rate before and after collection.
4. Submit at least one blank cassette per lot.
5. Avoid cross-contamination.





Questions?