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MODERN SAMPLING AND ANALYSIS TECHNIQUES

Ingeborg Bjorvand Engh,
PhD senior advisor Mycoteam AS

Konsulenter innen sopp- og insektspørsmål.
Biologiske bygningsskader.

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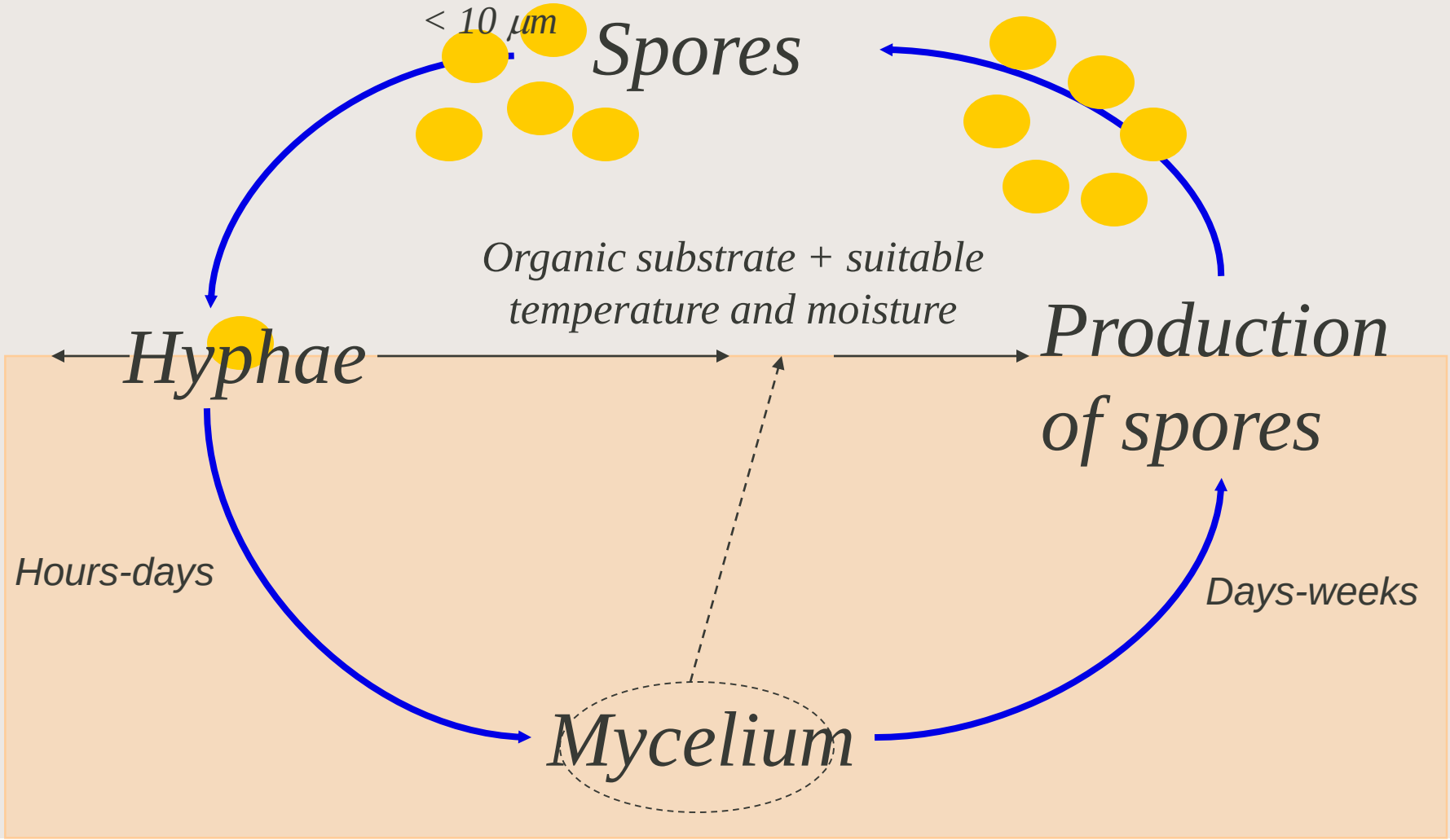
Hussopp

Life cycle moulds



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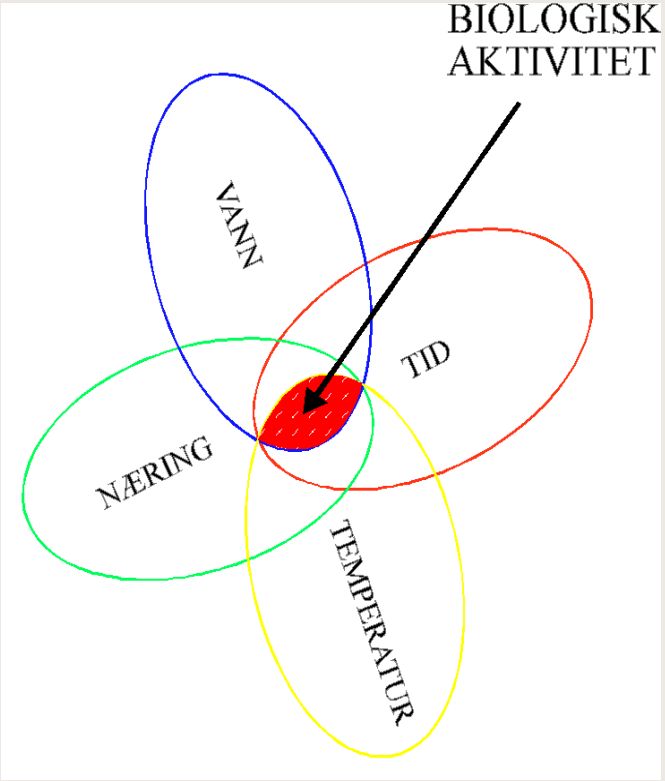
Biological activity



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Established sampling techniques

- Bulk material sampling
- Dust sampling
- Surface samples
- Air sampling
- Sampling fungal and microbial by-products

How to sample when there is nothing to see?

- No visible damage.
- No structural problems
- No aestetical issues
- Not detectable moisture
- No history of leakage, no old mould damages
- Noone can remember anything

Still – some have problems like asthma or allergy.

Limitations to traditional methods

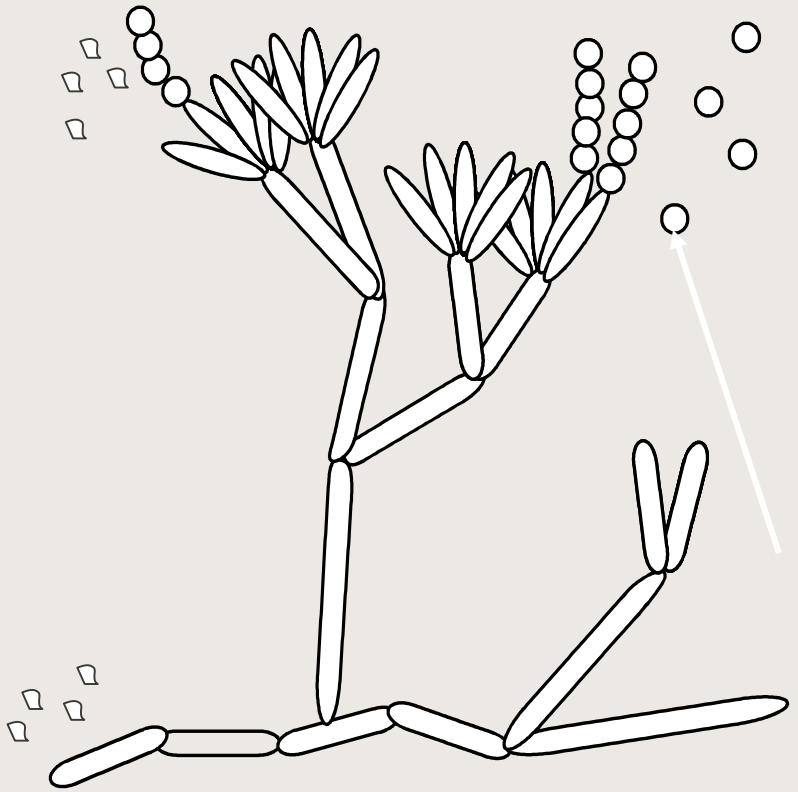
- Not all fungal genera or species are recognisable
- Small fragments and single spores are hard to detect
- Not all spores become airborne
- *Streptomyces*, *Mucor* and *Rhizopus* hard to find, but detected in most of the qPCR tests.
- Fungal genera and groups identified through direct microscopy were all detected using qPCR

Ask yourself…

- What is the problem?
- Where to take samples?
- How to sample?
- Interpretation of results:
  - To what extent or level
  - What should you tell?
  - Repairments necessary, remediation of damages
- Art of communicating
  - Communicating the risk
  - Telling the truth, but explain the facts too
    - «There are spiders»…

Bioactive substances and metabolites from moulds

- Spores
- Fragments of spores and hyphae
- Glucans
- Proteins
- Secondary metabolites including mycotoxins
-Microbial volatile organic compounds



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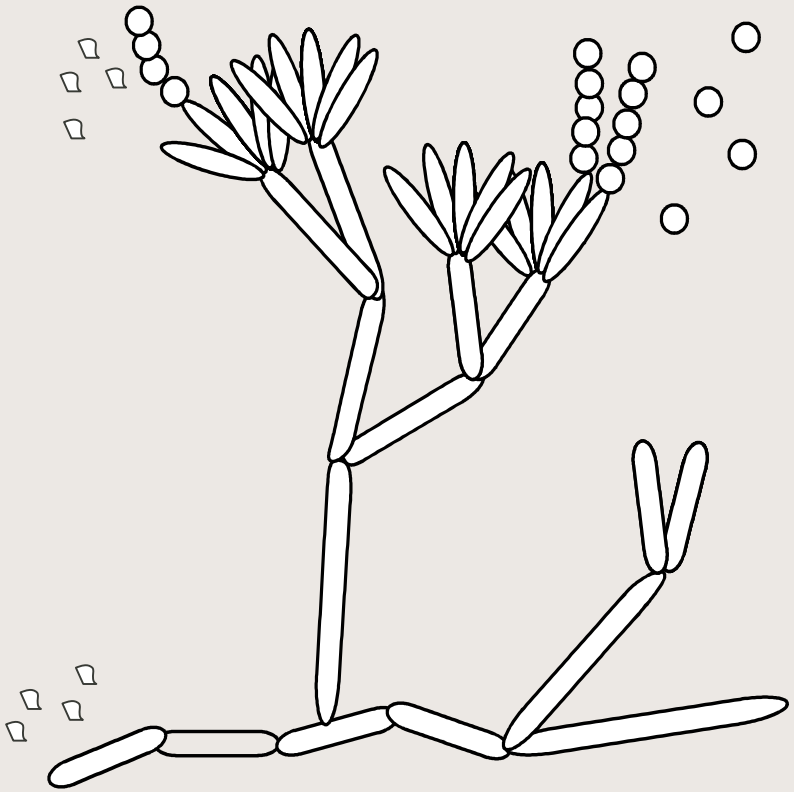
Mycotoxins

A mycotoxin is a metabolite that causes a toxic response when introduced by a natural route in low concentrations to higher vertebrates and animals



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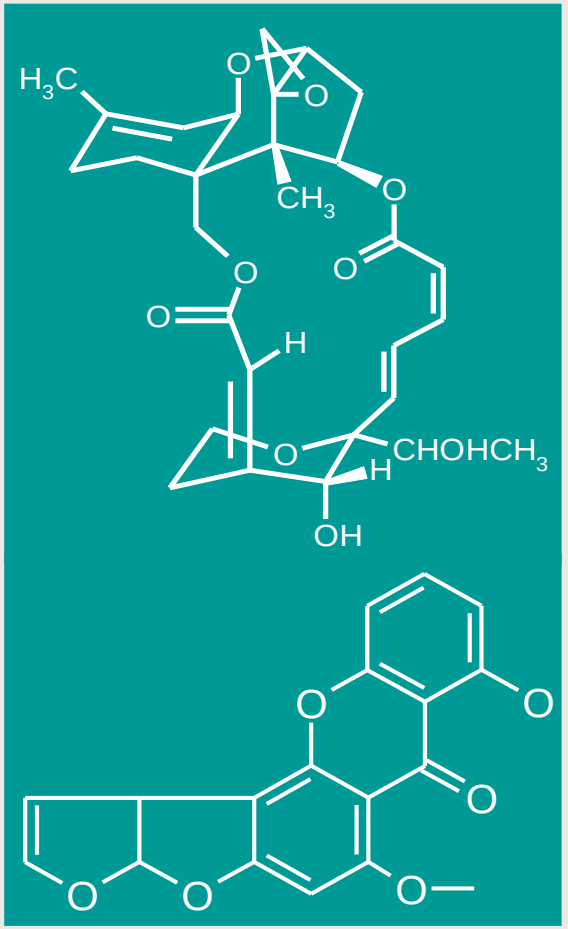
Microbial volatile compunds (MVOC)

- **Microbial volatile compunds (MVOC)**

- Microbial VOCs (mVOCs) are presently used as marker to detect human diseases, food spoilage or moulds in houses.

- no public and up-to-date collection on mVOCs is available

- Mycotoxins are not necessarily volatiles.



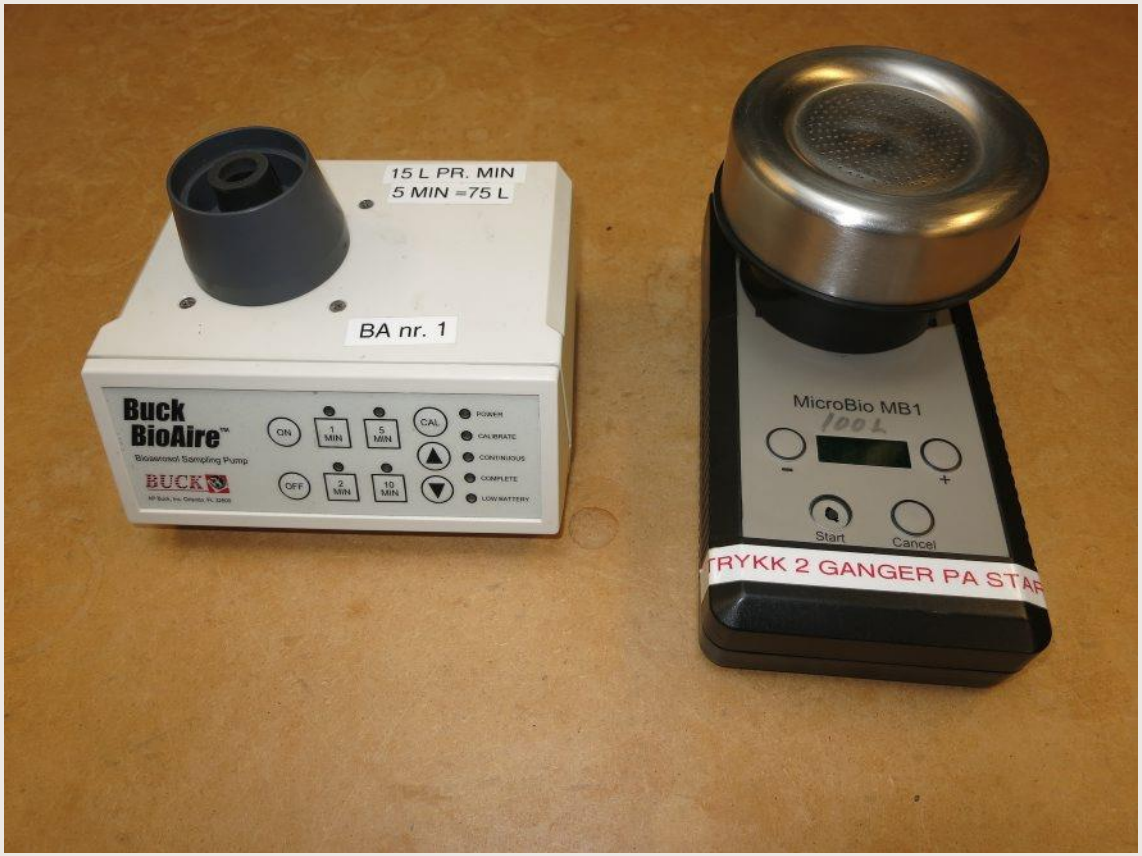
Mycometer test

- Rapid detection of fungi
- Analysis of fungi from air samples, surfaces etc.
- Verified by the US-EPA (Enviromental Protection Agency)
- In use in Europe and US

Applications Mycometer test

- Post remediation verification (PRV)
- Documenting the efficacy of the cleaning of surfaces for mould growth
- Documentation of mould growth
- Documentation that discoloration is NOT mould growth
- Delineation of mould growth where it is non-visible. How much should be cleaned?
- Documenting the cleaning of HVAC systems

Air sampling methods



Culturable, viable airborne moulds

- MicroBio Air Sampler
- Culture the fungi 1 week
- Results: Fungi identified to genus/species level
- Might reveal unknown or hidden damages
- Culturable viable spores ONLY
- Information from short time period

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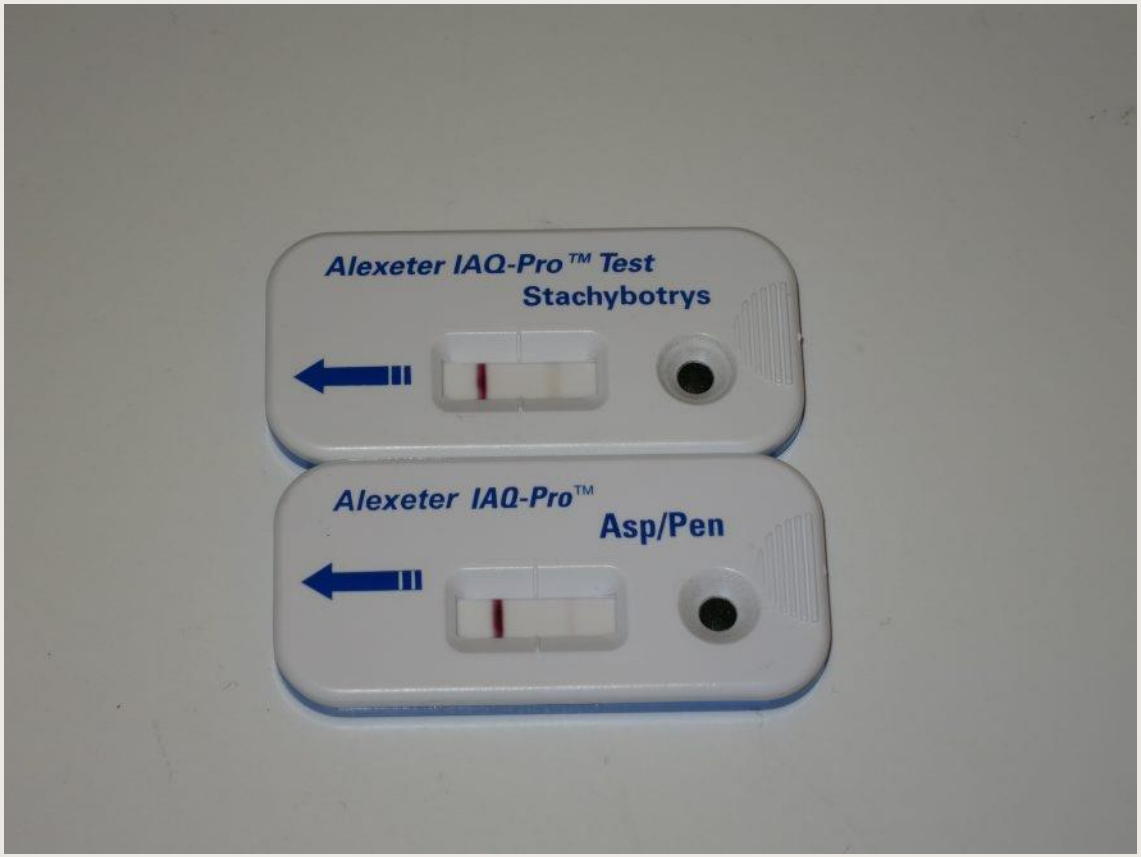
Impingement, impaction, filtration

- Liquid medium used for particle collection.
- Sampling possible directly on to Petri dishes.
- Sampling through a filter, a range of pore sizes available.

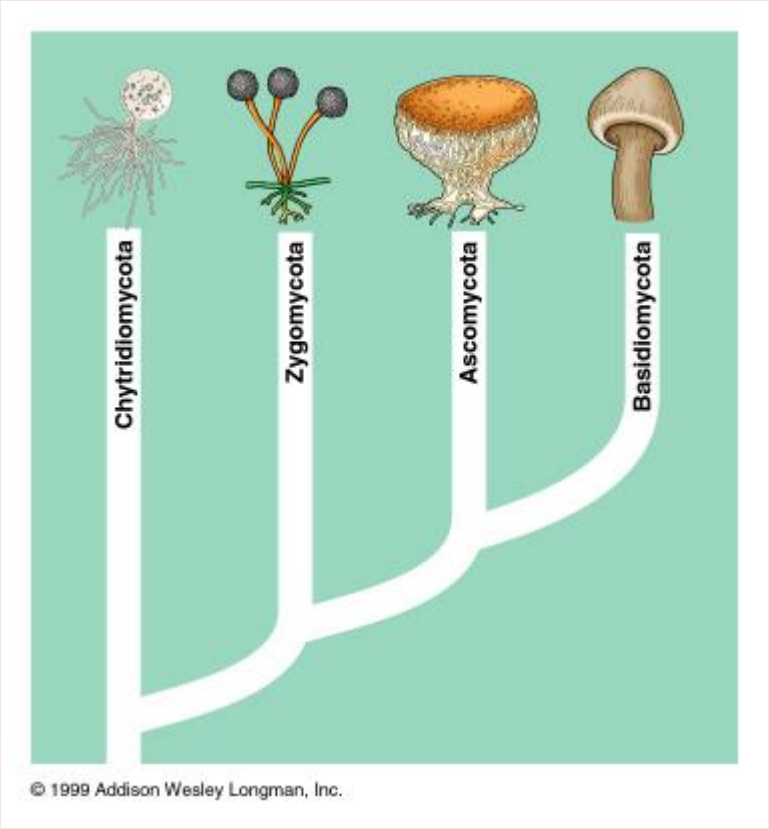

Alexeter IAQ-Pro™ Test
Stachybotrys
Alexeter IAQ-Pro™
Asp/Pen

Hyphal fragments

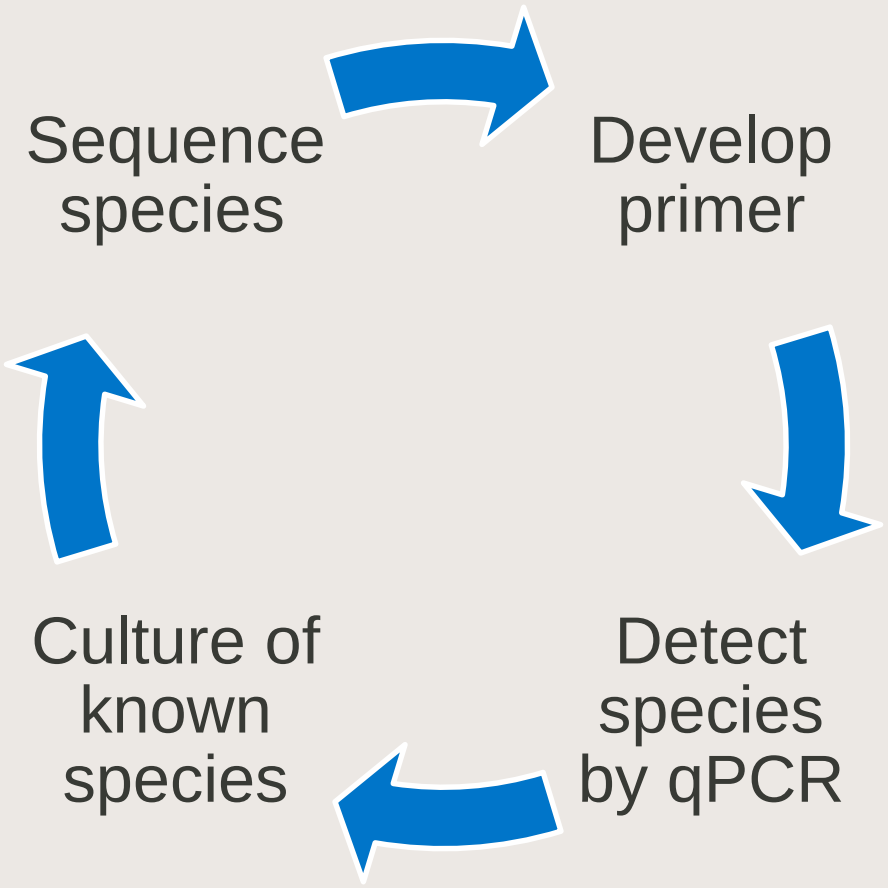
- Aerosolized fungal fragments are particles derived from any intracellular or extracellular fungal structure.
- Size: submicron particles or larger fungal fragments
- *In vitro* studies have shown that submicron particles of several fungal species are aerosolized in much higher concentrations (300–500 times) than spores.
- The combination of hyphal fragments and spore counts improved the association with asthma severity.
- Higher respiratory deposition than spores.
- Detection by immunodetection techniques

DNA based techniques

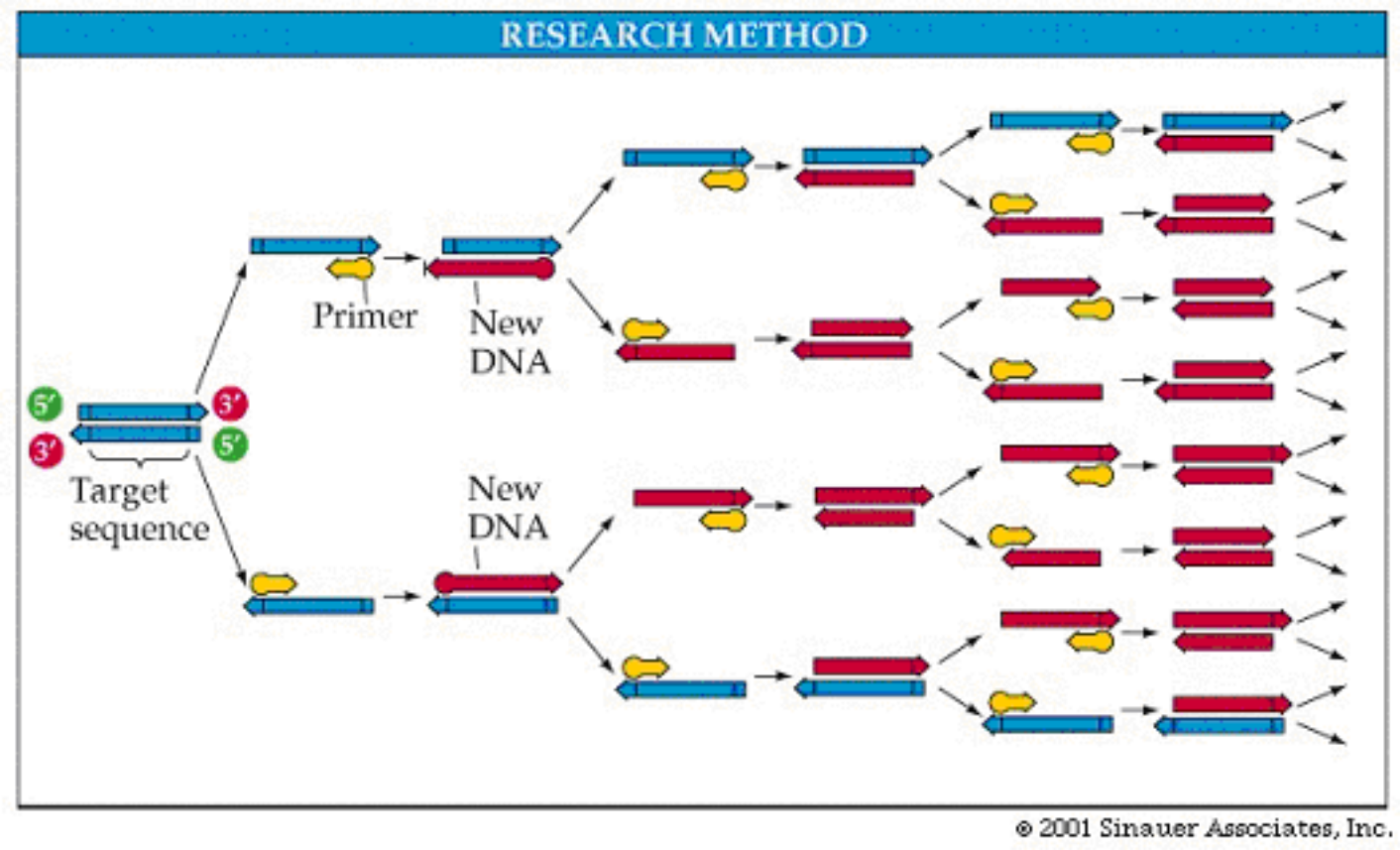
- Where can we find DNA?
  - Cells: Spores, hyphae
  - Extracellular DNA
- How much do we need?
- How can we use DNA to identify a species?
- When to use DNA for identification?



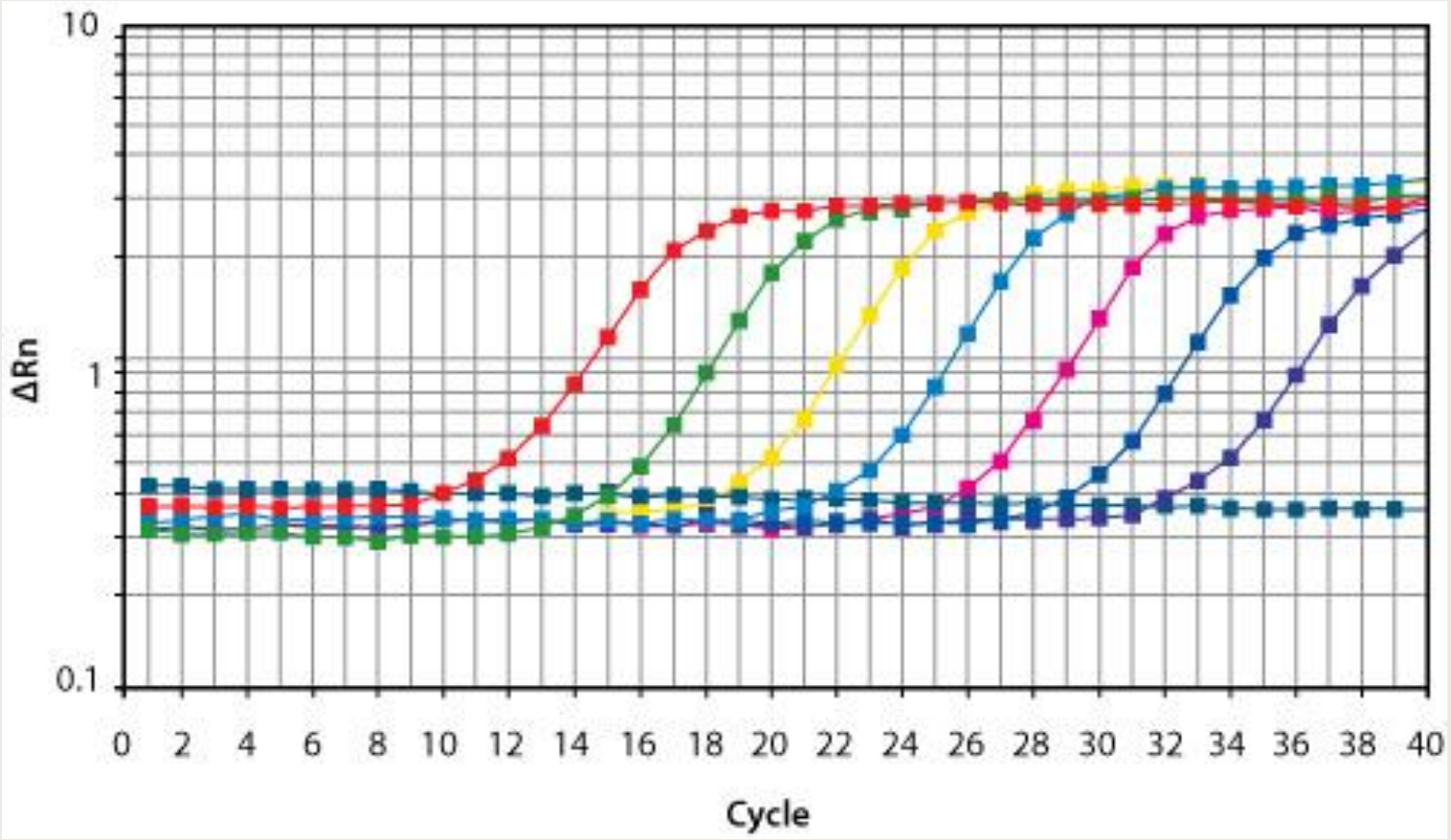
Identification starts with identification…



PCR reaction principle



Quantification of strain in test tube



DNA tests available

- qPCR several vendors all over Europe and especially US labs
- ERMI test (**EPA Technology for Mold Identification and Enumeration)**
  - A DNA-based analysis called Mold Specific Quantitive PCR (MSQPCR) of 36 molds including, the 26 Group 1 species associated with homes with water damage and the 10 Group 2 species which are found in homes independent of water damage, forms the basis of the ERMI.
- Several vendours in Europe

Fungal genera and species - primers

- **Primers and Probes for Target Species**
- *Absidia* | *Acremonium* | *Alternaria* | *Aspergillus* | *Aureobasidium* | *Candida* | *Chaetomium* | *Cladosporium* | *Emericella* |*Eurotium* | *Epicoccum* | *Fusarium* | *Geotrichum* | *Memnoniella* | *Mucor* | *Myrothecium* | *Paecilomyces* | *Penicillium* |*Rhizomucor* | *Rhizopus* | *Scopulariopsis* | *Stachybotrys* | *Trichoderma* | *Ulocladium* | *Wallemia* | Universial *Penicillium, Aspergillus, Paecilomyces* |

Type of substrate

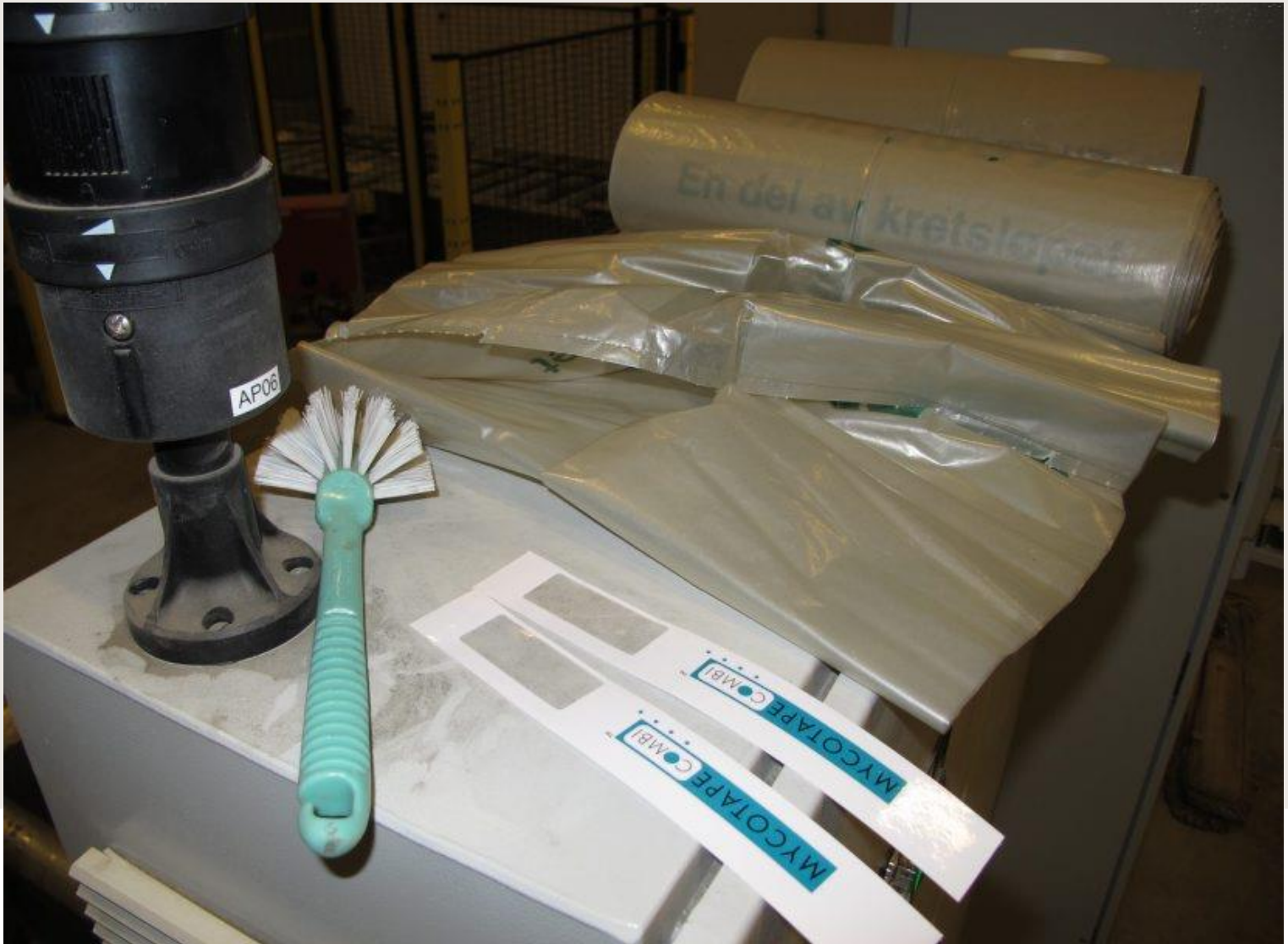
- Surfaces
- Air samples
- Vacuum samples
- Dust samples
- Bulk samples

Different levels of cell equivalents present in differing samples.

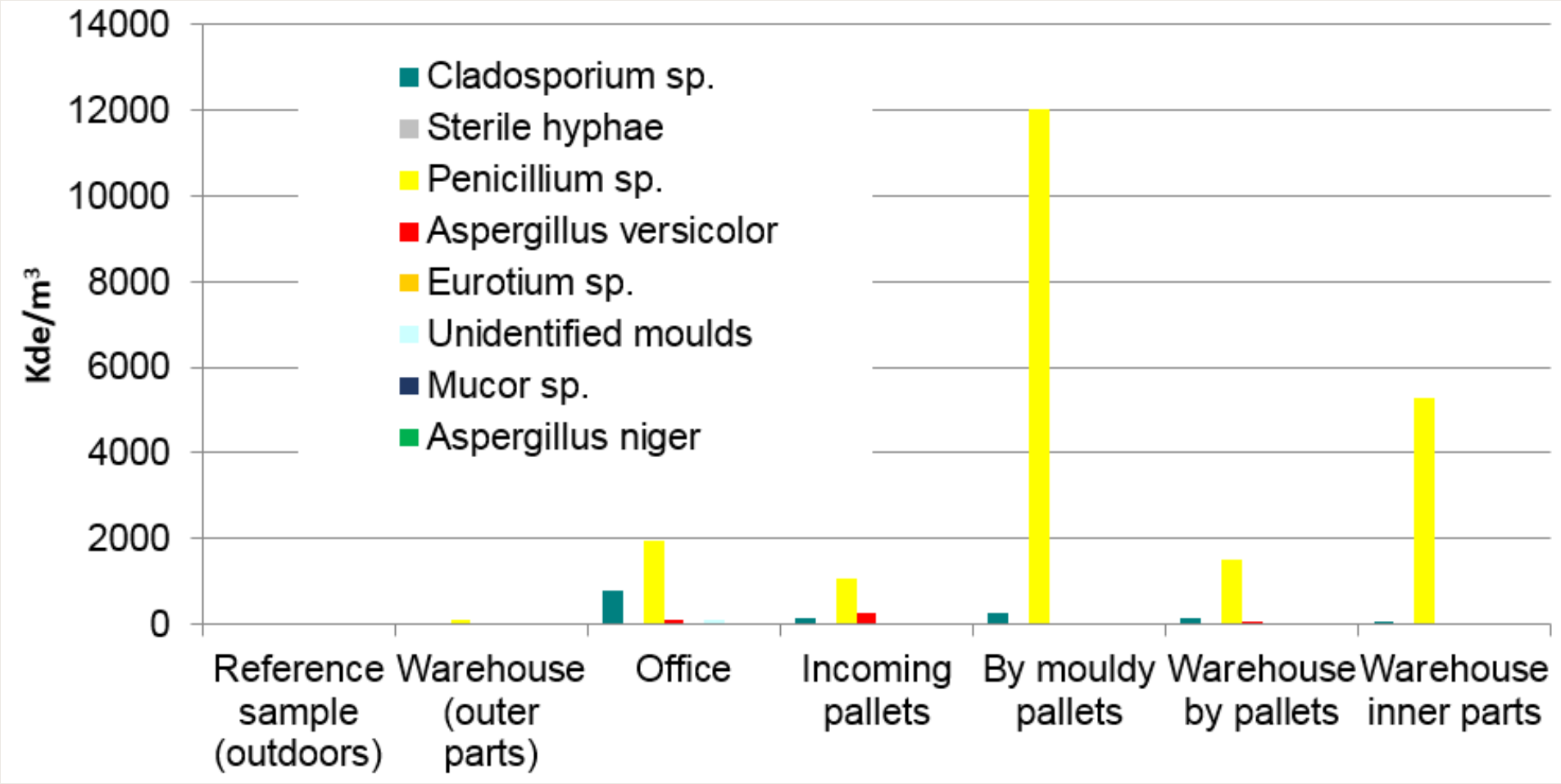
***Penicillium* sp.**
***Cladosporium* sp.**

Sampling dust from surfaces

- qPCR test of dust sampled by tape lifts



Viable airborne spores – cultured



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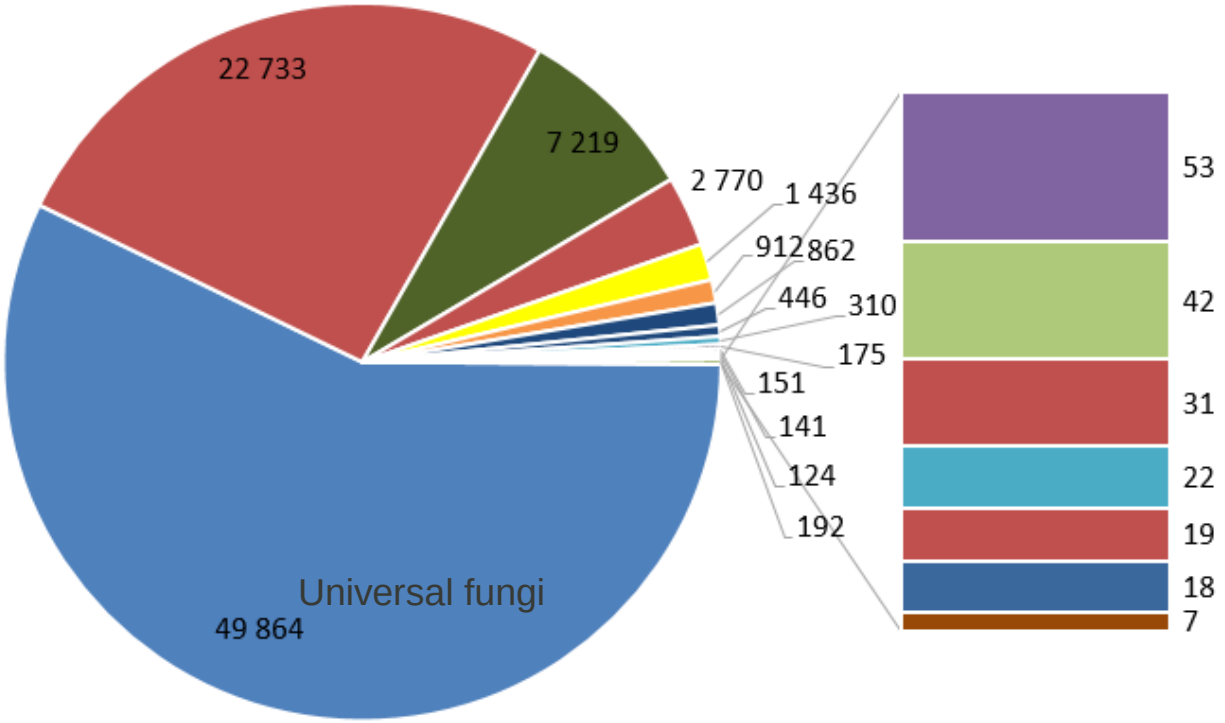
qPCR results

20 groups of fungi quantified



- Universal fungi
- *Trichoderma viride*
- *Streptomyces*
- *Cladosporium cladosporioides*
- *Mucor/Rhizopus*
- *Cladosporium sphaerospermum*
- *Aspergillus fumigatus*
- *Alternaria alternata*
- *Rhizopus stolonifer*
- *Stachybotrys chartarum*
- *Penicillium/Aspergillus/Paecilomyces*
- *Aspergillus versicolor*
- *Wallemia sebi*
- *Cladosporium herbarum*
- *Acremonium strictum*
- *Aspergillus glaucus*
- *Chaetomium globosum*
- *Penicillium chrysogenum*
- *Aspergillus niger*
- *Ulocladium chartarum*

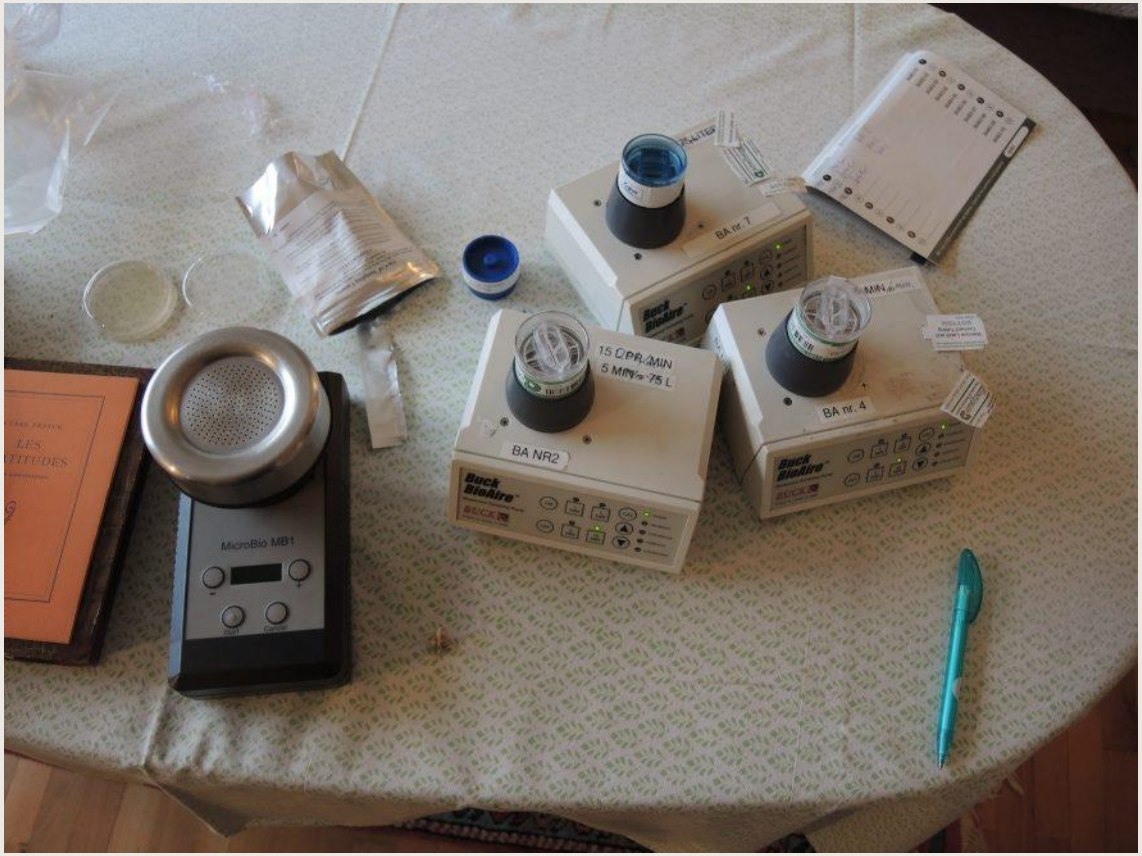
Fungi in surface dust samples

- *Chaetomium globosum* identified through microscopy.
- Typically the genera *Alternaria*, *Aspergillus*, *Penicillium* and *Cladosporium*
- Basidiomycetes, Ascomycetes

Eleven to twenty different groups of fungi were identified employing the qPCR technique

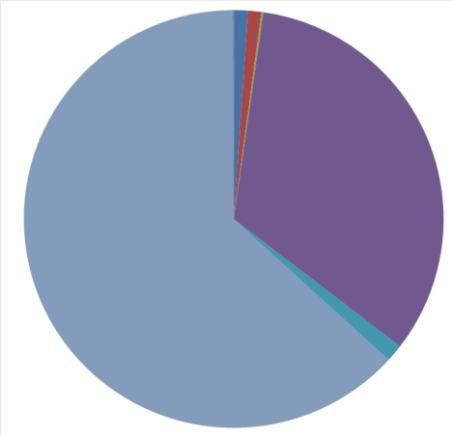
Table 1. Number of species/groups of taxa identified in dust and air samples represented by cases.

Case name	Dust samples number of species		Air samples number of species	
	Microscopy	qPCR	Microscopy	qPCR
Private home A	5	15	-	-
Private home B	2	14	2	12
School building R	5	7	1	7
School building K	-	7	-	9
Private home C	2	15	-	16

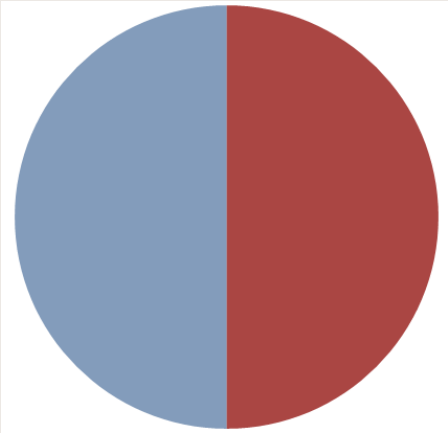

MicroBio MB1
BA NR2
BA nr. 4
Buck BioAire


X RLU
1285
Overflate rengjøring
SystemSURE Plus
OK
P
i
hygiena

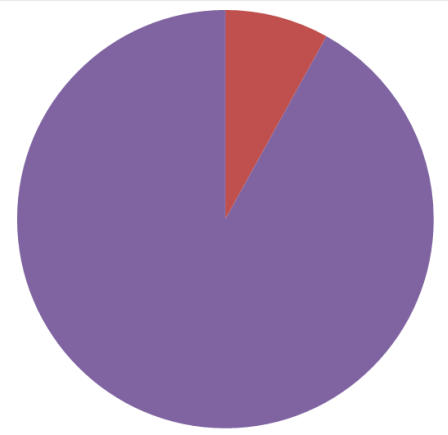
School building



A) qPCR



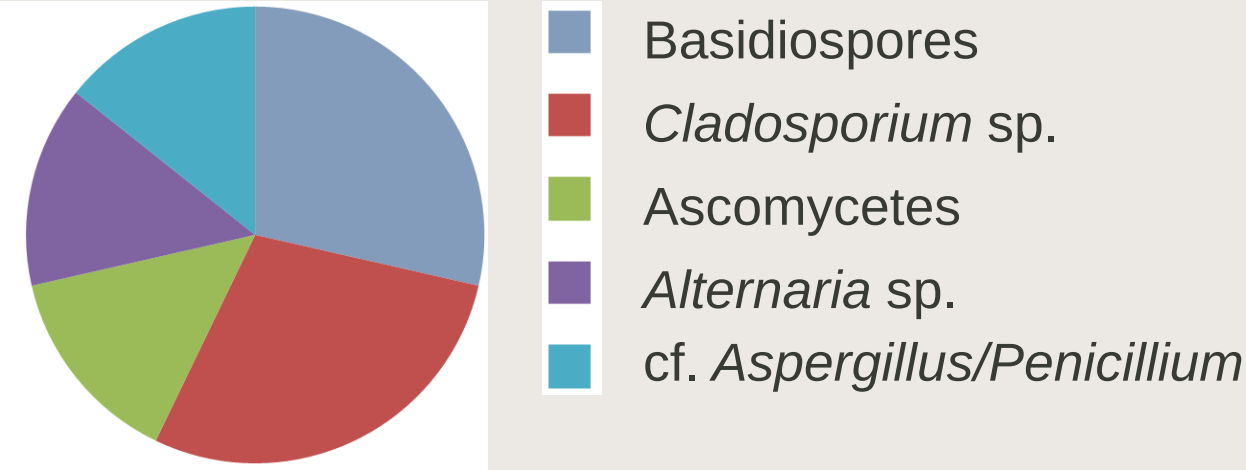
B) Total spore count



C) Viable spore count

- *Aspergillus niger*
- *Cladosporium cladosporioides*
- *Cladosporium herbarum*
- *Penicillium/Aspergillus/Paecilomyces*
- *Aspergillus versicolor*
- Universal fungi
- *Mucor/Rhizopus*
- *Wallemia sebi*

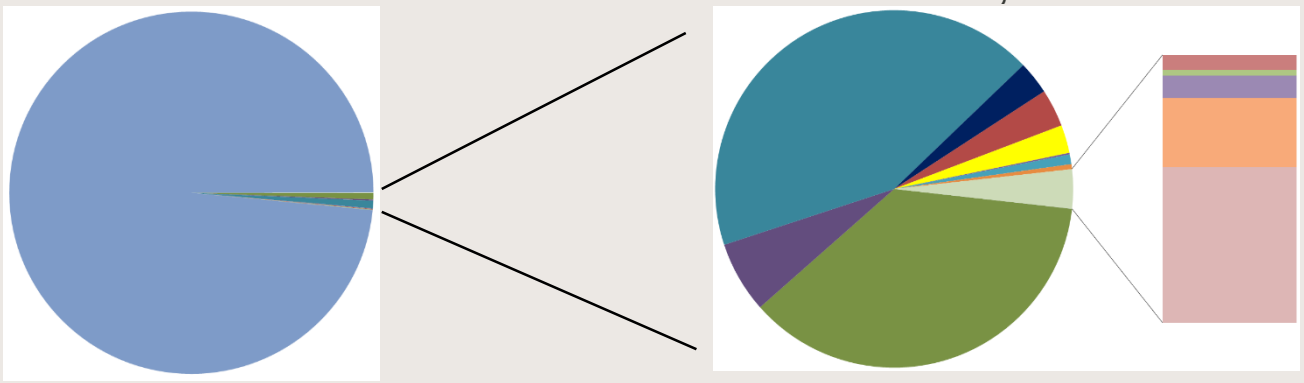
Private home – Dust samples



Direct microscopy of dust and mould spores from horisontal surface in bedroom, private home. Percent dust coverage was ranging from 22.3 % to 32 %.

Private home

324,653 detected spore equivalents



- *Cladosporium cladosporioides*
- *Cladosporium herbarum*
- *Penicillium/Aspergillus/Paecilomyces*
- *Streptomyces*
- *Aspergillus versicolor*
- *Acremonium strictum*
- *Alternaria alternata*
- *Cladosporium sphaerospermum*
- *Aspergillus glaucus*
- *Mucor/Rhizopus*
- *Penicillium chrysogenum*
- *Rhizopus stolonifer*
- *Trichoderma viride*
- *Wallemia sebi*

Applications qPCR tests

- Control after remediation
- Documenting the efficacy of cleaning of surfaces after mould damage, or control of cleaning in general
- Air movements: Does spore spread throughout building?
- Documentation that discoloration is NOT mould growth
- Delineation of mould growth where it is non-visible. How much should be cleaned?

Limitations to DNA based methods

- Expensive
- Loss of information on
  - Dead or living spores/hyphae
  - Dust and particles in the sample

- No information if mould lacks air-borne spores
- Yet DNA seems to be present even in hyphal fragments
- Best result when interpreted with information about moisture, dust etc.

How to complement qPCR?