



Detectie en identificatie van schimmels in het binnenmilieu en de werkomgeving

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Overview

- Introduction
- Recommended approaches
- methods for isolation and detection



Ecology of indoor moulds

- The fungal flora of indoor environments is known and is different in its composition from outdoors (phyllloplane fungi)
- The fungal flora is very similar to that occurring in food
- many species are xerotolerant or xerophilic
- species are specific for materials with different wateractivities





List of fungal species occurring in indoor environments

<i>Absidia corymbifera</i>	<i>Curvularia lunata</i>	<i>Penicillium olsonii</i>
<i>Acremonium murorum</i>	<i>Emericella nidulans</i>	<i>Penicillium rugulosum</i>
<i>Acremonium strictum</i>	<i>Epicoccum nigrum</i>	<i>Penicillium simplicissimum</i>
<i>Alternaria alternaria</i>	<i>Eurotium amstelodami</i>	<i>Penicillium spinulosum</i>
<i>Aspergillus candidus</i>	<i>Eurotium chevalieri</i>	<i>Penicillium variabile</i>
<i>Aspergillus clavatus</i>	<i>Eurotium herbariorum</i>	<i>Phialophora fastigiata</i>
<i>Aspergillus flavus</i>	<i>Exophiala dermatitidis</i>	<i>Phialophora verrucosa</i>
<i>Aspergillus flavipes</i>	<i>Fusarium culmorum</i>	<i>Phoma glomerata</i>
<i>Aspergillus fumigatus</i>	<i>Fusarium solani</i>	<i>Phoma macrostoma</i>
<i>Aspergillus niger</i>	<i>Fusarium verticillioides</i>	<i>Pithomyces chartarum</i>
<i>Aspergillus ochraceus</i>	<i>Geomyces pannorum</i>	<i>Pyronema domesticum</i>
<i>Aspergillus penicillioides</i>	<i>Geotrichum candidum</i>	<i>Rhizopus stolonifer</i>
<i>Aspergillus restrictus</i>	<i>Memnoniella echinata</i>	<i>Rhodotorula mucilaginosa</i>
<i>Aspergillus sydowii</i>	<i>Mucor hiemalis</i>	<i>Schizophyllum commune</i>
<i>Aspergillus terreus</i>	<i>Mucor plumbeus</i>	<i>Scopulariopsis brevicaulis</i>
<i>Aspergillus versicolor</i>	<i>Mucor racemosus</i>	<i>Scopulariopsis candida</i>
<i>Aureobasidium pullulans</i>	<i>Oidiodendron griseum</i>	<i>Scopulariopsis fusca</i>
<i>Botrytis cinerea</i>	<i>Oidiodendron rhodogenum</i>	<i>Serpula lacrymans</i>
<i>Candida peltata</i>	<i>Paecilomyces lilacinus</i>	<i>Sistotrema brinkmannii</i>
<i>Chaetomium aureum</i>	<i>Paecilomyces variotii</i>	<i>Sporobolomyces roseus</i>
<i>Chaetomium globosum</i>	<i>Penicillium</i>	<i>Stachybotrys chartarum</i>
<i>Chaetomium indicum</i>	<i>aurantiogriseum</i>	<i>Syncephalastrum racemosum</i>
<i>Chrysonillia sitophila</i>	<i>Penicillium</i>	<i>Trichoderma harzianum</i>
<i>Cladosporium</i>	<i>brevicompactum</i>	<i>Trichoderma koningii</i>
<i>cladosporioides</i>	<i>Penicillium chrysogenum</i>	<i>Trichoderma viride</i>
<i>Cladosporium herbarum</i>	<i>Penicillium citrinum</i>	<i>Tritirachium oryzae</i>
<i>Cladosporium</i>	<i>Penicillium commune</i>	<i>Ulocladium chartarum</i>
<i>sphaerospermum</i>	<i>Penicillium corylophilum</i>	<i>Verticillium lecanii</i>
<i>Clonostachys rosea</i>	<i>Penicillium expansum</i>	<i>Wallemia sebi</i>
<i>Coprinus cordisporus</i>	<i>Penicillium glabrum</i>	
<i>Cryptococcus laurentii</i>	<i>Penicillium janthinellum</i>	

Toxinogenic – pathogenic – xerophilic - basidiomycete

Approach for examination

- Always start with a good inspection of the house or building
- Note water, moisture, leakage problems
- Inform about the history of the house or building
- Collect materials



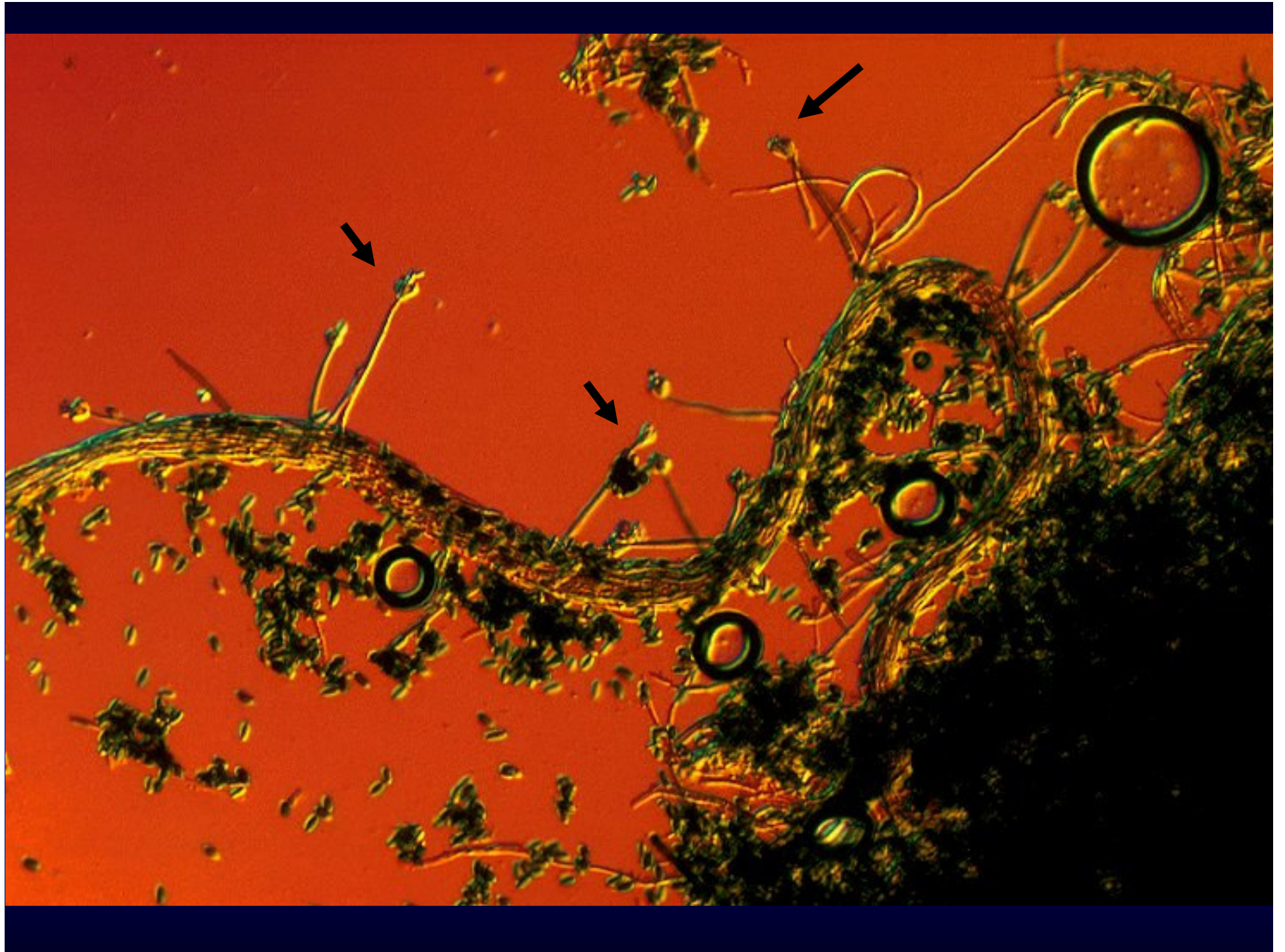
Material for sampling

- Building materials
- Scraping of the wall
- House dust
- Cellotape preparations

Scotch tape preparation

- Transparent Scotch or cello tape
- Stick the adhesive part to the area you like to investigate
- Stick it to a microscope slide
- In the laboratory place a drop of lactic acid with some aniline blue between the slide and the tape
- Examine in the microscope





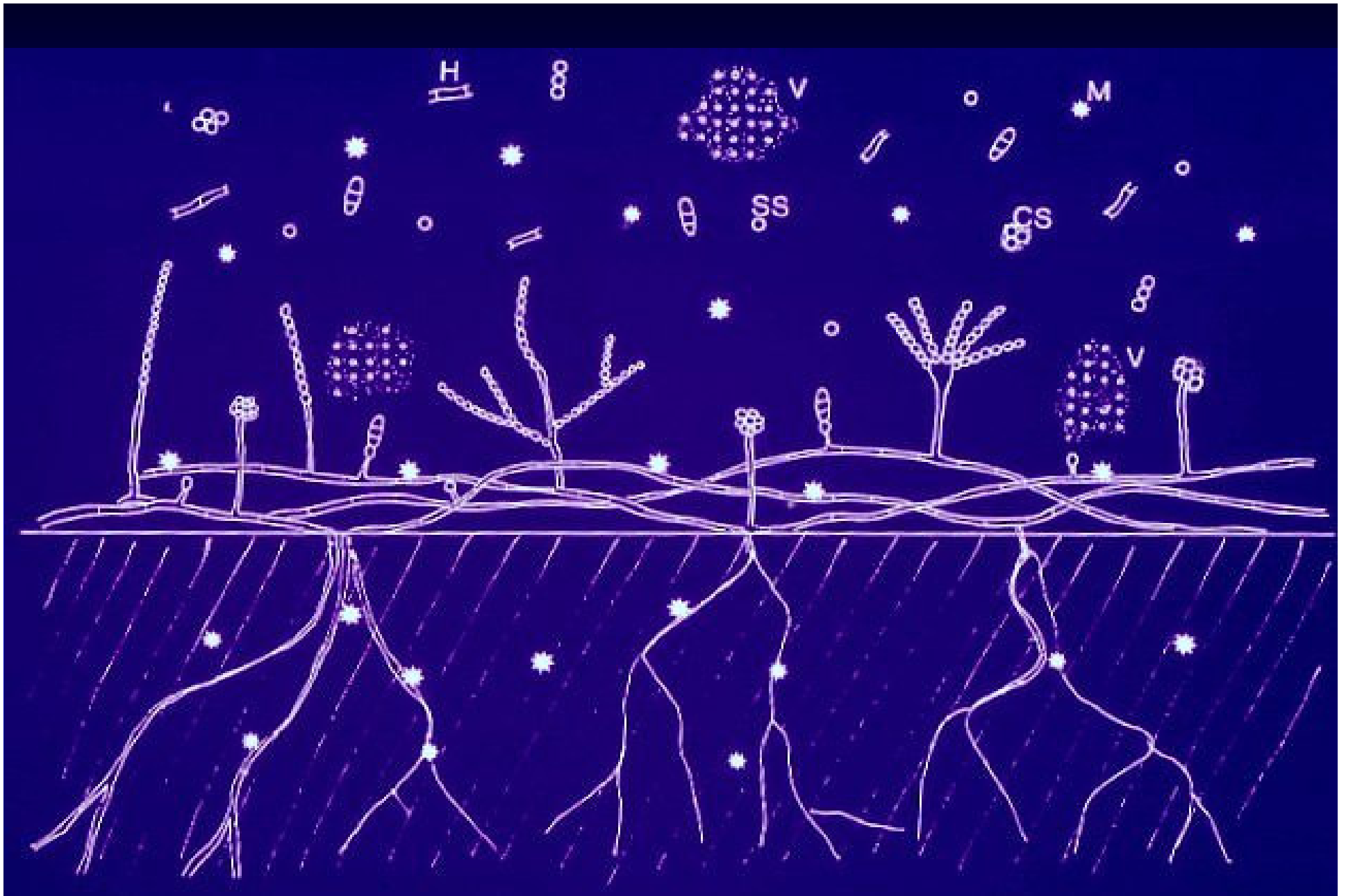
Detection of indoor moulds

- Microscopic examination of materials (cellotape preparations)
- swabs of surfaces
- direct plating
- airsampling

What is in the air?

- Fungal spores/conidia (2-10 μm)
- Various fungal fragments (pieces of mycelium, fruit-bodies etc.) (<0.1-2.5 μm)
- Fungal metabolites
 - Primary metabolites
 - Secondary metabolites: mycotoxins, volatiles
 - Metabolites can also be in the spores and mycelium





Fungal propagules and products which may become airborne

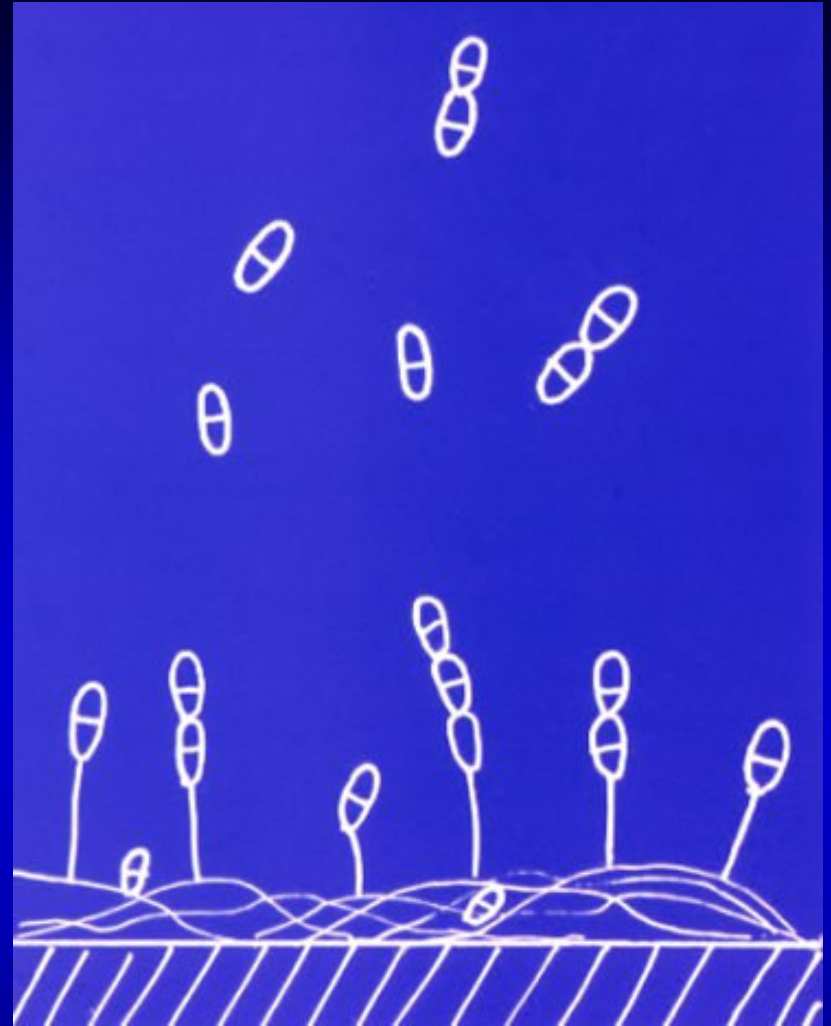
Air sampling

- Non volumetric (e.g. Open Petri dish). This is not very suitable because the spores in the air differ in size and weight
- Volumetric sampler.
 - There are various types and brands
 - There is not very much difference in the results of the various samplers
 - Important is that the equipment is reliable and that the correct media are used
 - The equipment should be handy and practical e.g. battery operated



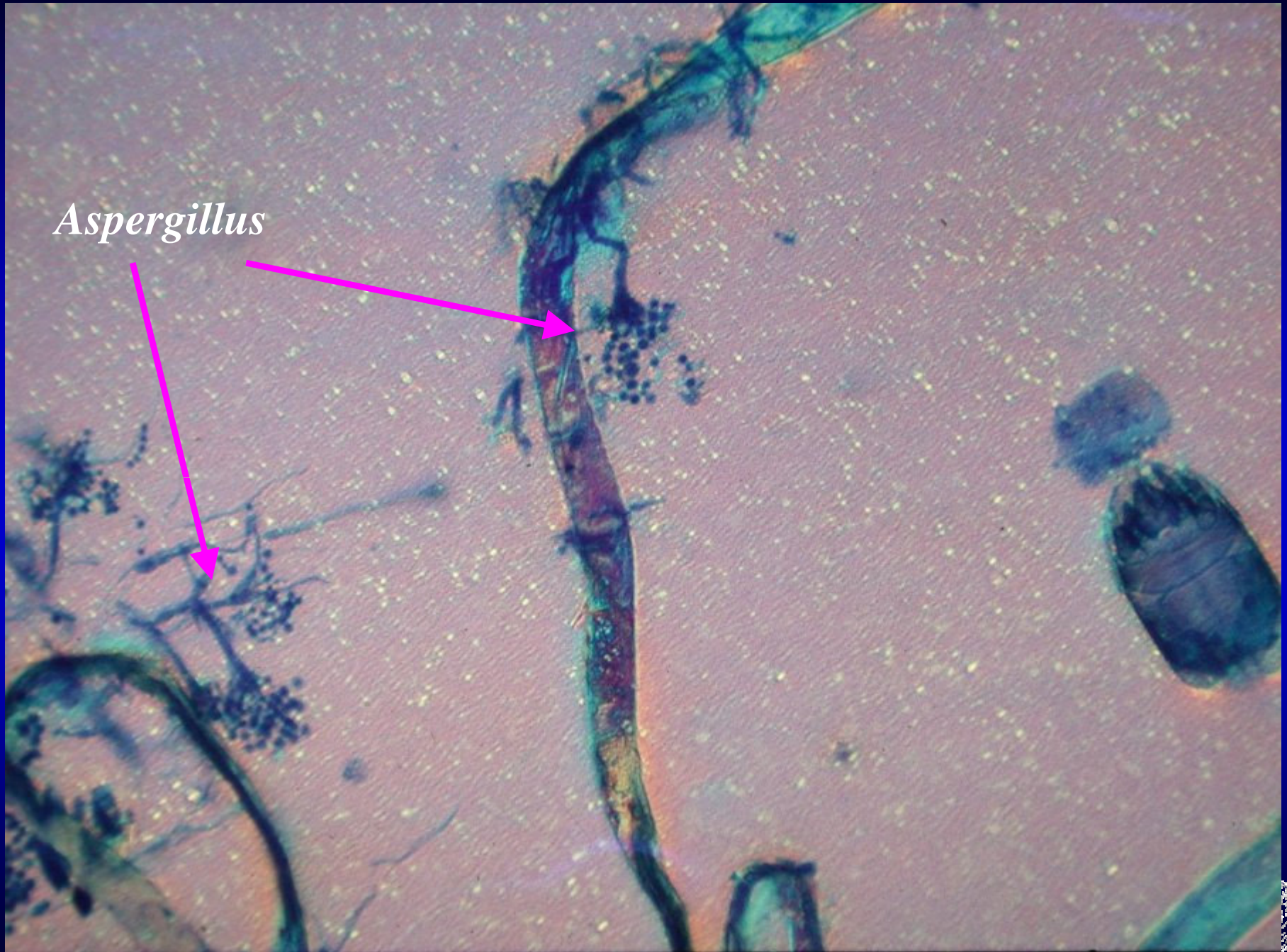


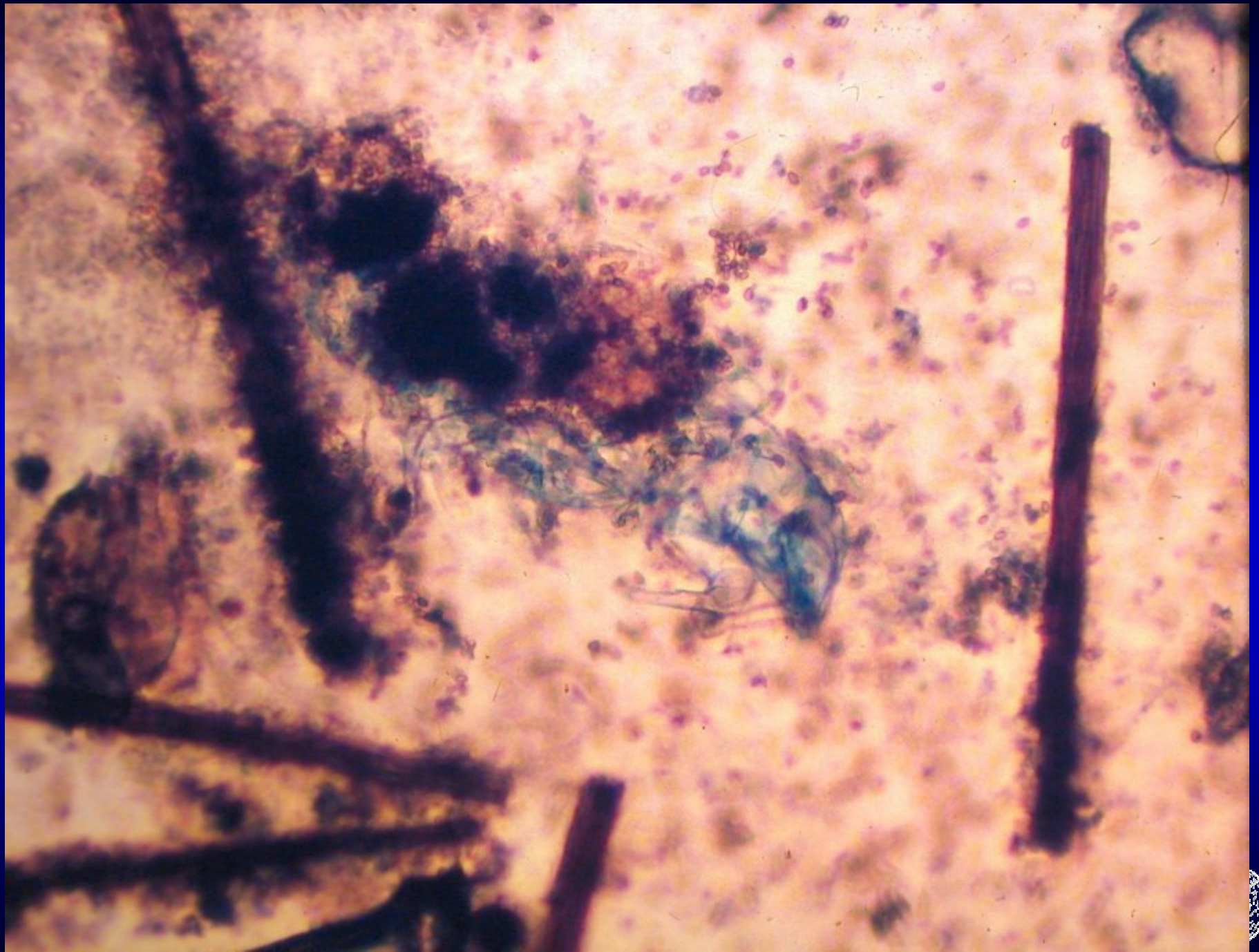
One *Aspergillus* conidiophore producing numerous conidia



Alternaria colony with abundant mycelium but only with few conidia

Aspergillus





Quantitative estimation of airborne moulds

- Mould colonies can produce many different propagules which become airborne
- There is much difference in the way and the degree of sporulation
- Spores may occur singly, in chains, in groups or in slimy aggregates
- In samples mostly the viable propagules can be detected
- The number of airborne propagules vary in time and depends on factors such as wind, movement etc.



Detection: media and incubation

- Media
 - moist and wet environments:
 - malt extract agar
 - dry environments:
 - DG18 agar
 - malt extract agar
- Incubation
 - temperature: 25°C (or higher)
 - time: 5 days for counting, longer for identification



Bacteria

MEA

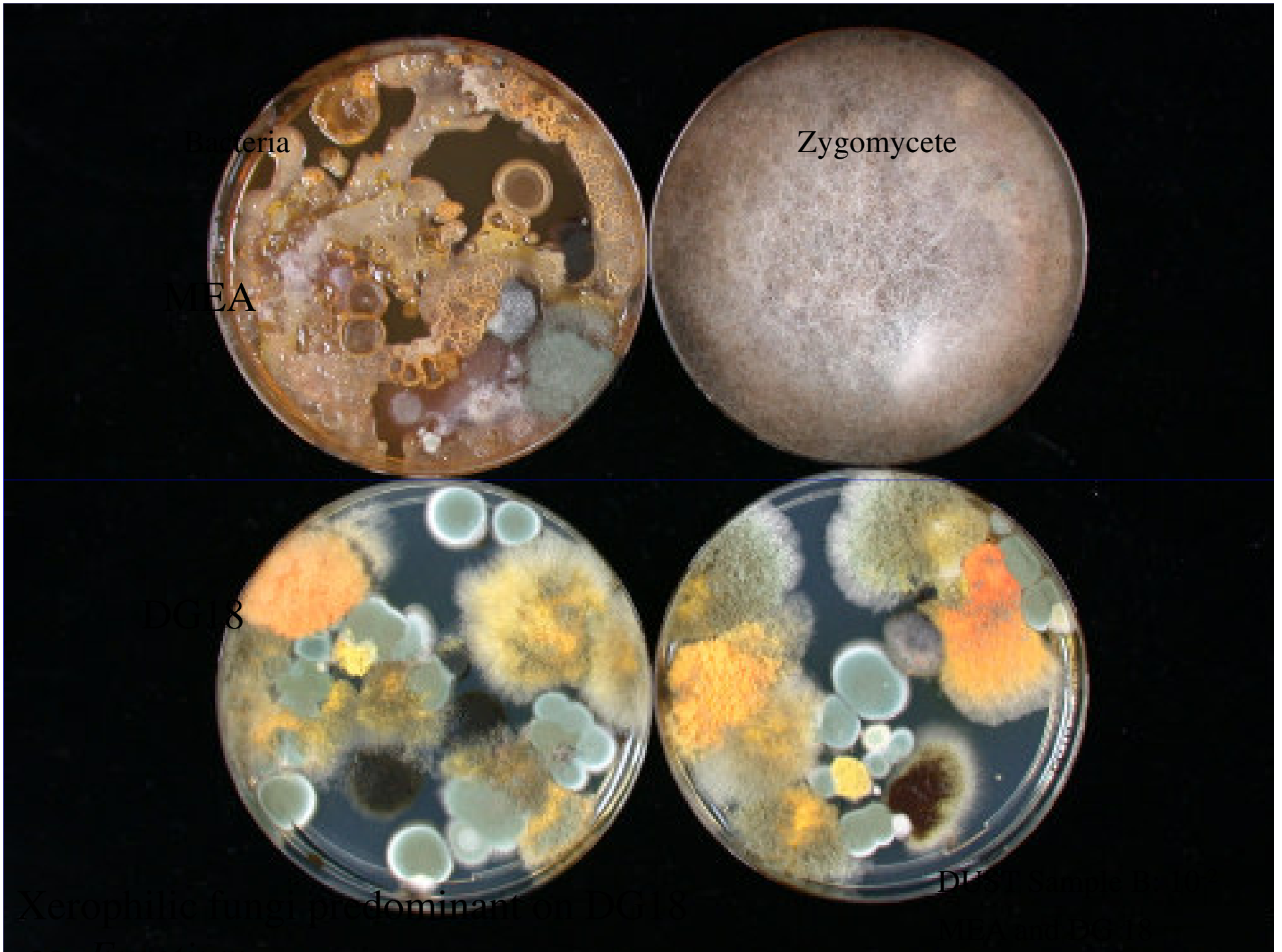
Zygomycete

DG18

Xerophilic fungi predominant on DG18

DG18 Sample B

MEA and DG18



Identification of the fungi

- The identity of the species will reveal important information:
 - Dominance
 - Pathogenicity
 - Toxicity
 - Conditions of growth



Summary

- There are no standards for measuring fungi in indoor environments
- Most methods only detect viable fungal propagules and the significance of dead fungal fragments should not be underestimated
- Quantitative estimation can provide an indication but is not a precise method
- Identification of the fungi is important

Symposium

- Fungi and Health
- 13 en 14 november 2008
- Trippenhuis KNAW Amsterdam
- Sessie on Living in healthy environments
 - sprekers: Flannigan, Warscheid, Adan & Samson
- www.cbs.knaw.nl

