

NEMC



Premise Microbial Testing

Advanced IAQ Microbial Sampling Methods

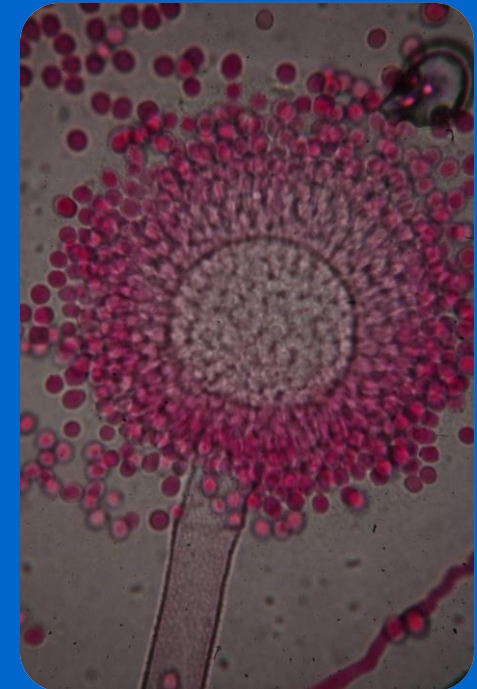


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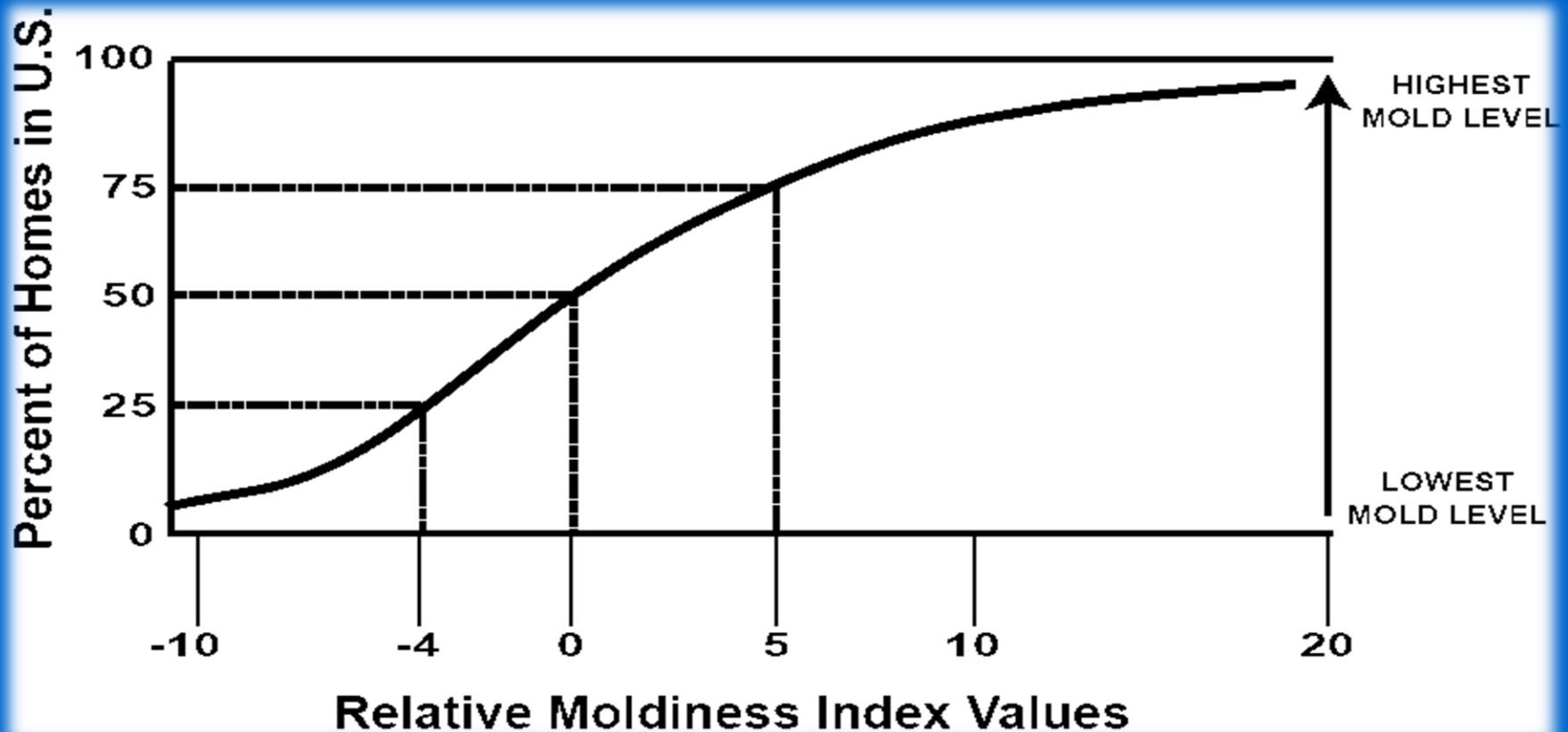
ERMI

- Environmental Relative Moldiness Index
- EPA developed PCR technology for >100 fungi
- Pilot study on dust in moldy and non-moldy homes
- Narrowed down to 36 fungi
- HUD dust collection study (>1000 homes)



Aspergillus

ERMI Across US



Source : Dr. Steve Vesper, EPA



Dust Sampling

- Composite sample from living room and bedroom
- Tape off a 3 by 6 foot area in each location
- Vacuum for ~5 mins in each location
- Problematic if:
 - No dust around
 - New carpets
 - Newly steamed carpets
 - Rugs used in multiple locations



Dust Sampler


Lab Sample Number	Client Sample ID	Sample Location	Sample Size	EMSL Order#
371200720-123	Client-1	Family Rm/Bdrm	5 mg	371200000

Environmental Relative Moldiness Index (ERMI) by Mold Specific Quantitative Polymerase Chain Reaction (MSQPCR)

Lab Sample Number	371200720-123
Client Sample ID	Client-1
Sample Location	Family Rm/Bdrm
Sample size	5mg Dust
EPA 36 Species Identification Group 1	Spores E./ mg dust
<i>Aspergillus flavus</i>	ND
<i>Aspergillus fumigatus</i>	ND
<i>Aspergillus niger</i>	4,832
<i>Aspergillus ochraceus</i>	ND
<i>Aspergillus penicillioides</i>	195
<i>Aspergillus restrictus</i>	ND
<i>Aspergillus sclerotiorum</i>	447,846
<i>Aspergillus sydowii</i>	ND
<i>Aspergillus unguis</i>	ND
<i>Aspergillus versicolor</i>	ND
<i>Eurotium (A.) amstelodami</i>	ND
<i>Aureobasidium pullulans</i>	928
<i>Chaetomium globosum</i>	1,461
<i>Cladosporium sphaerospermum</i>	1
<i>Paecilomyces variotii</i>	ND
<i>Penicillium brevicompactum</i>	ND
<i>Penicillium corylophilum</i>	ND
<i>Penicillium crustosum (group2)</i>	ND
<i>Penicillium purpurogenum</i>	ND
<i>Penicillium spinulosum</i>	ND
<i>Penicillium variable</i>	ND
<i>Scopulariopsis brevicaulis</i>	ND
<i>Scopulariopsis chartarum</i>	50
<i>Stachybotrys chartarum</i>	ND
<i>Trichoderma viride</i>	ND
<i>Wallemia sebi</i>	ND
Group 1 Sum of the Logs	19.5



Lab Sample Number	371200720-123	
Client Sample ID	Client-1	
Sample Location	Family Rm/Bdrm	
Sample size	5mg Dust	
EPA 36 Species Identification Group 2	Spores E./ mg dust	
<i>Acremonium strictum</i>	16	
<i>Alternaria alternata</i>	4	
<i>Aspergillus ustus</i>	ND	
<i>Cladosporium cladosporioides I</i>	20	
<i>Cladosporium cladosporioides II</i>	ND	
<i>Cladosporium herbarum</i>	ND	
<i>Epicoccum nigrum</i>	669	
<i>Mucor and Rhizopus group</i>	ND	
<i>Penicillium chrysogenum</i>	ND	
<i>Rhizopus stolonifer</i>	ND	
Group 2 Sum of the Logs	5.9	<input checked="" type="checkbox"/>

Interpretation Key Group 2 Sum of the Logs

	≤ 2 or ≥ 20 -- Less than or equal to 2 or Greater than or equal to 20
<input checked="" type="checkbox"/>	> 2 and < 20 -- Greater than 2 or Less than 20

ERMI Value:	14	
ERMI Interpretation*	Level 4	

Interpretation Key ERMI Value

	ERMI Value > 5 -- High Relative Moldiness. Further Investigation needed to determine the sources of this mold.
	ERMI Value > 4 and ≤ 5 -- Moderate Relative Moldiness; Further investigation needed to determine if sources of mold exists.
<input checked="" type="checkbox"/>	ERMI Value ≤ 4 -- Low Relative Moldiness. It is unlikely you have a mold problem.

Office of Inspector General Letter

- Public May Be Making Indoor Mold Cleanup Decisions Based on EPA Tool Developed Only for Research Applications
- Not validated for public use by EPA
- Not “EPA-approved” method



Specific molds associated with asthma

- an 80% likelihood of finding an asthmatic child in a home with an ERMI value of about 1 or greater.



Vesper SJ, et al. *J Occup Environ Med.* 2006;48:852–858

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Mold species in dust from the International Space Station identified and quantified by mold-specific quantitative PCR

- Dust from HEPA filters in the U.S. Laboratory Module of the International Space Station (ISS).
- 39 molds found
 - Potential opportunistic pathogens and potential moderate toxin producers found



Vesper, SJ, Wong, W, Kuo, CM & Pierson, DL. Research in Microbiology Vol. 159, no. 6, pp. 432-435. Jul-Aug 2008.

Sewage Bacteria

- Fecal contamination from back-ups or pipe leaks for grey or black water events
- Culturable sewage screen using swabs
 - Total Coliform
 - *E. coli*
 - Enterococci



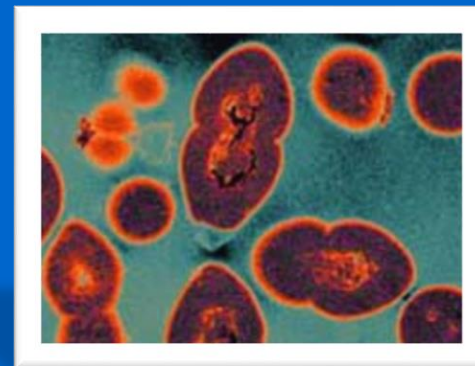
Occurrence of Feces Specific Indicator Bacteria

per g feces

- *E. coli* 10^7 - 10^9
- *Enterococci* 10^5 - 10^6
- *Bacteroides* 10^{11}



Escherichia coli



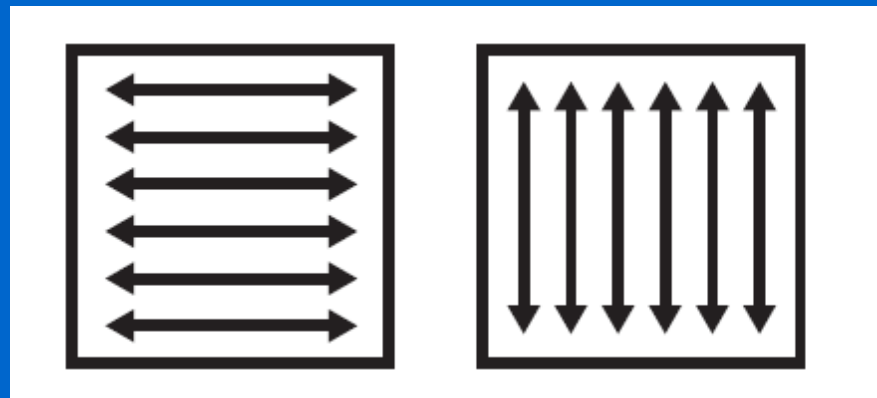
Enterococci

Bacteroides as Indicators

- PCR, no culturing
- More specific than culture indicators
- More sensitive than culture indicators
- Total or Human
- Anaerobic

Surface (Swab) Sampling

- Use a sterile, pre-moistened swab
- 4" by 4" grid template for each sample to be taken
- An alternative would be to use electrical tape to mark out a 4" by 4" grid and then swab inside that area.
- Rotate the swab 180 degrees to present a fresh swab surface between the latitudinal and the start of the longitudinal passes.



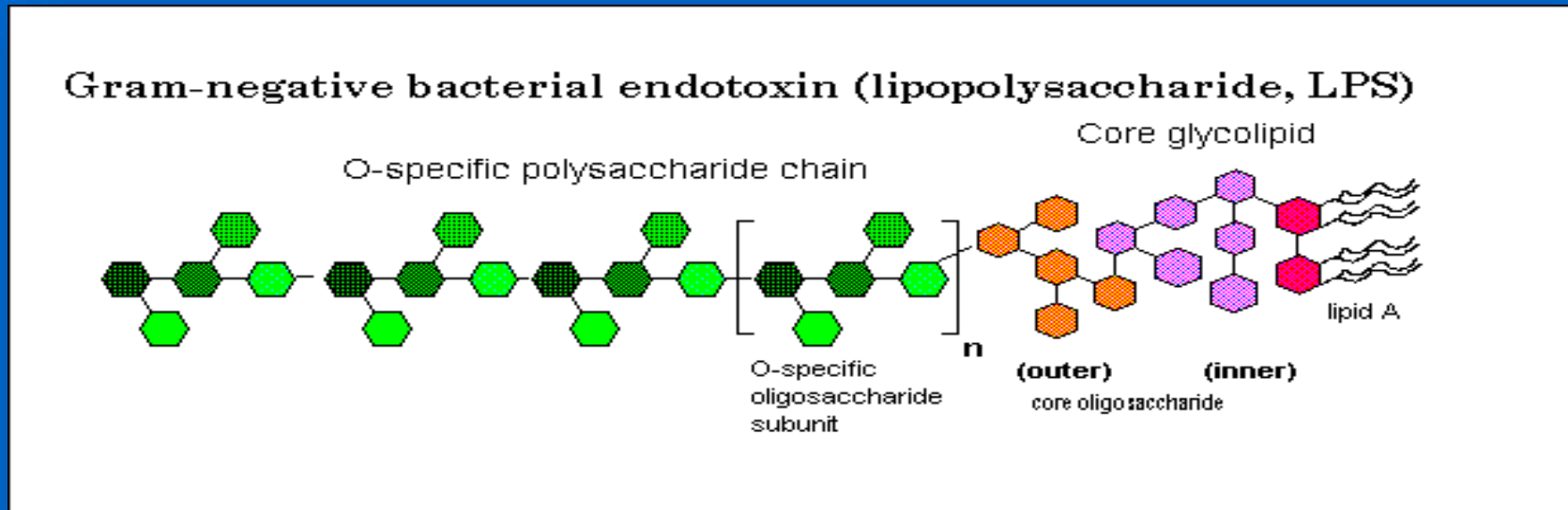
Bulk Sampling

- Bulk samples of carpet, wall board, clothing may also be taken (2" x 2")
- Clean cutting knives or scissors before use



Endotoxin

- Pyrogenic, cell wall component (lipopolysaccharide) of Gram-negative bacteria.
- Heat-stable



Health Effects

- Produces airway inflammation (wheezing)
- Associated with increased severity of child and adult asthma
- Exposure during childhood may reduce allergic responses later in life
- Cause of Humidifier Fever



- 'sick building syndrome' to be six to seven times higher than those in control buildings (254 vs. 46 ng/m³)



Materials & Methods

- Specifications:
 - 0.45µm Polycarbonate Filter
 - 37mm Styrene Housing
 - Sterilized by Irradiation
 - Endotoxin Free
- Sift dust through 350 micron sieve
- Kinetic Chromogenic *Limulus* amoebocyte lysate (LAL) assay (EU – endotoxin units)



Horseshoe Crab Harvest



Blood Collection

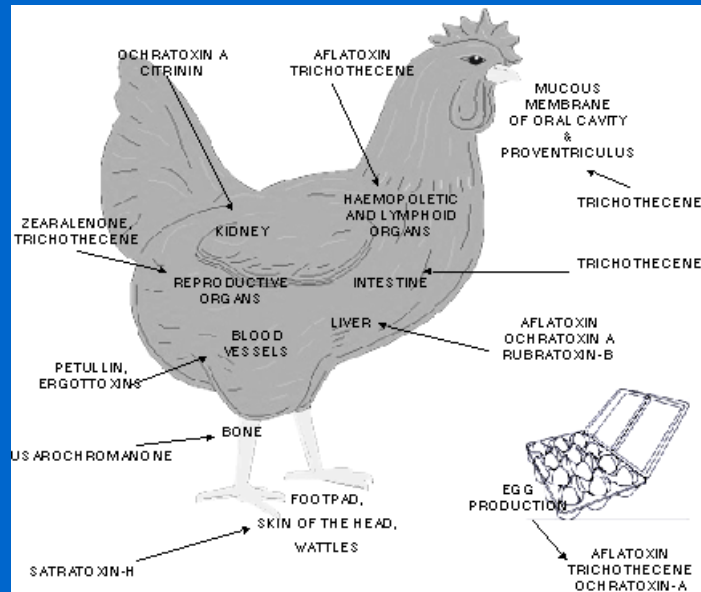


Endotoxin Predictors in Literature

- Positively correlated with presence of a dog or past presence of a dog
- Positively correlated with use of a cool-mist humidifier
- Negatively correlated with use of a dehumidifier & central air conditioning
- Poor home hygiene

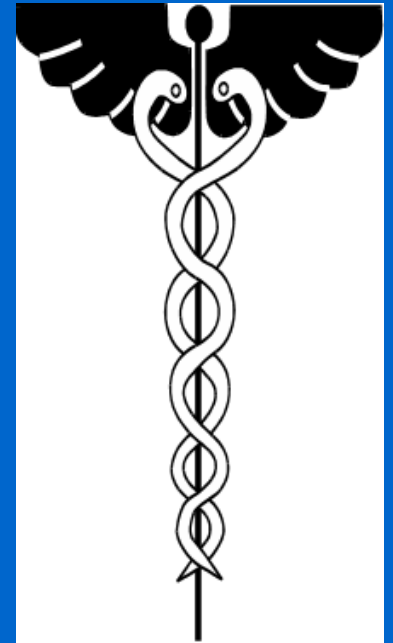


MYCOTOXINS



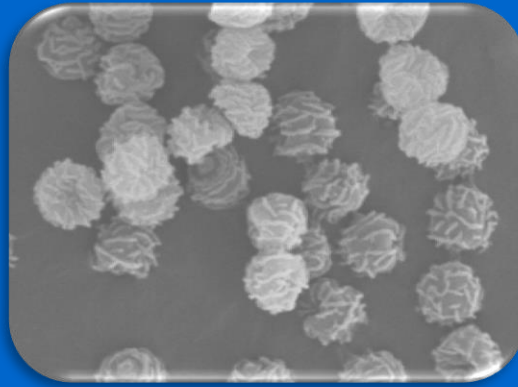
Mycotoxins

- Some fungi are known to produce toxic metabolites
- Toxic, carcinogenic, estrogenic, or immuno-suppressive
- Majority of fungi have not even been screened for mycotoxins (400-500 known)



They can be found in

- fungal spores



- contaminated substrates



- mycelia



Mycotoxin Sampling

- Bulk material (square foot)
- Swabs (3 or more from same area)
- Air samples are difficult
 - SpinCon PAS 450-10 (450 Lpm)
 - Andersen GPS-1 (PUF) (150 Lpm)



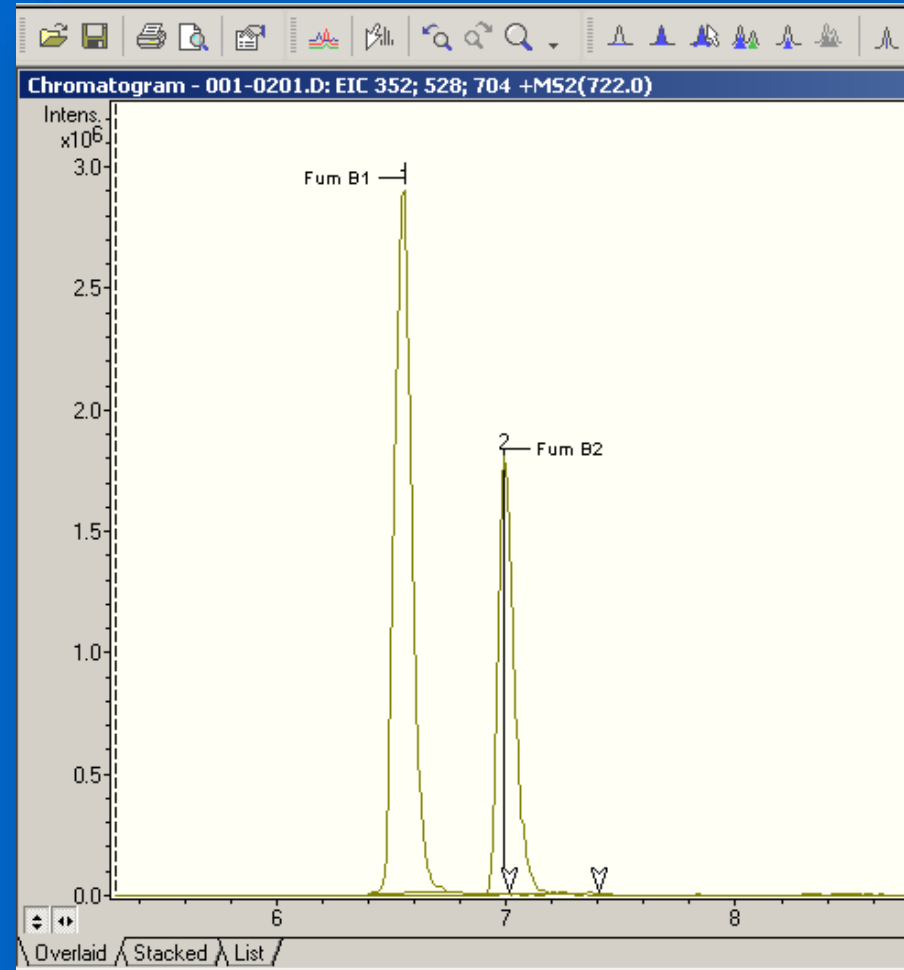
<https://www.rkb.us/contentimages/63274.jpg>

Detection of Airborne *Stachybotrys chartarum* Macrocytic
Trichothecene Mycotoxins in the Indoor Environment
T. L. Brasel, J. M. Martin, C. G. Carriker, S. C. Wilson, and D.C. Straus



Mycotoxin Analysis

- Trichothecenes (*Stachybotrys* toxins)
- Aflatoxin (*Aspergillus* spp.)
- Ochratoxin (*Aspergillus* spp. & *Penicillium* spp.)
- Many more available by ELISA, VICAM, or LC-MS Methods



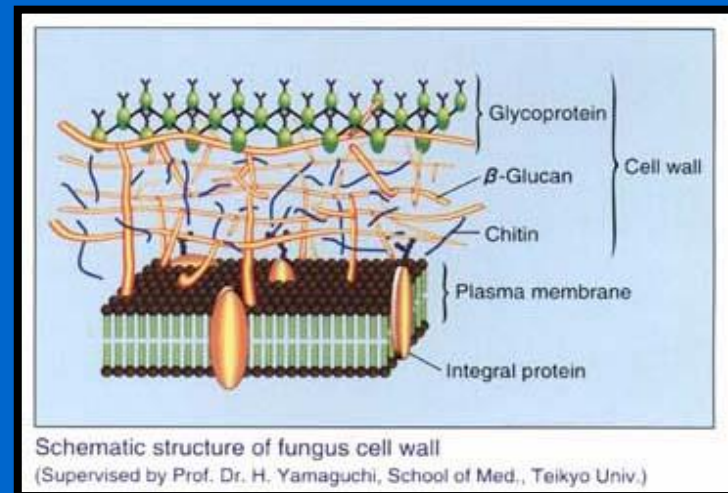


Glucans



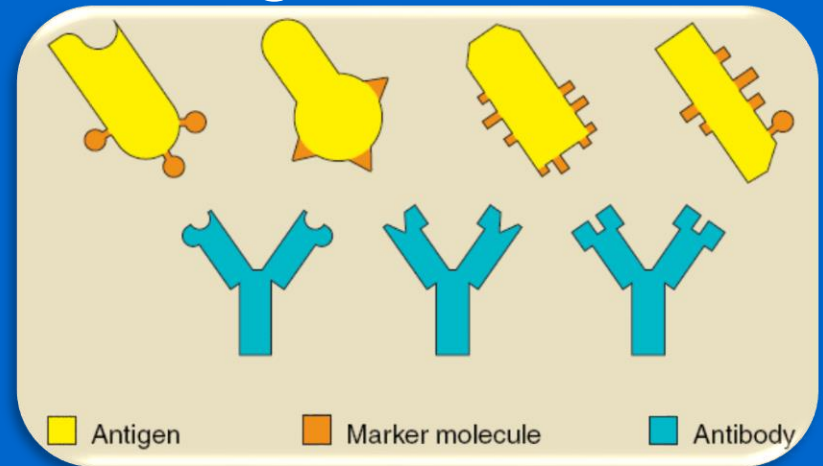
$(1 \rightarrow 3)\beta$ -D-glucan

- β -D-Glucans
 - part of the cell wall of the majority of fungi (some bacteria and some plants also)
 - pyrogenic (causes fever)
- Used to measure fungal biomass (living or dead)



Glucans

- Taken up by white blood cells and digested over several weeks.
- Causes inflammation
- Increased reactivity to antigens
- Granulomas



Sampling Methods

- Settled dust on surfaces or airborne dust



Particle Breakdown

- Glucan Percentage
 - 30% spores
 - 30% hyphae and spore fragments
 - 40% in finer, unrecognizable particulates
- Reference: JD Miller 2007



New Orleans Study

- After hurricane Katrina and Rita
- Indoor geometric mean = 1700 ng/m³
- Outdoor geometric mean = 900 ng/m³
- Mod/Heavy mold damaged homes = 1800 ng/m³



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Questions?

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