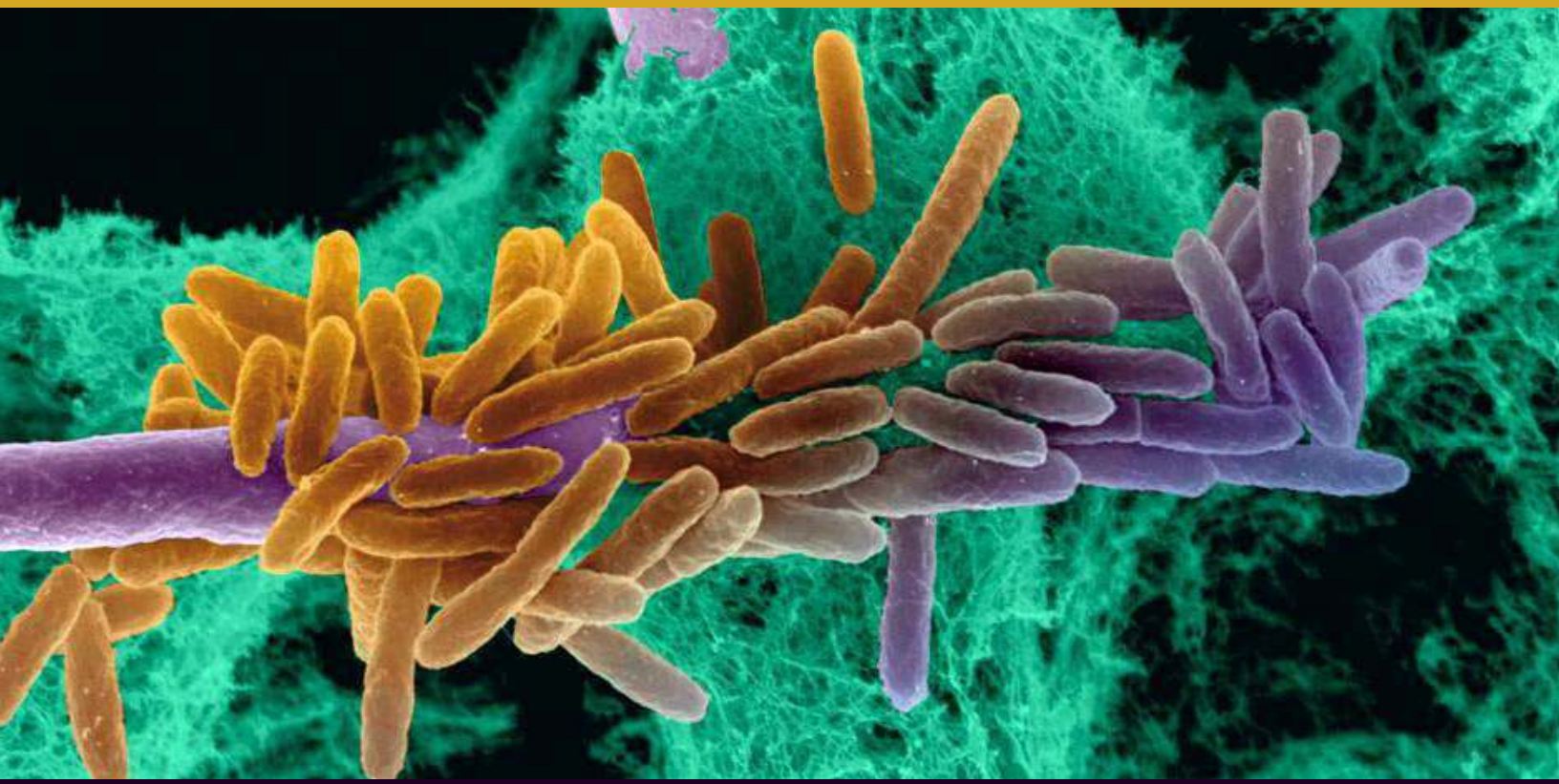
PART 3: CASE STUDIES: WHAT WORKS OR NOT WITH MOLD REMEDIATION AND TESTING

SURVIVING MOLD ILLNESS

Why are so many mold-sensitive patients not getting better?



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CASE STUDIES PART 3

Surviving Mold Illness PART 1 RECAP

- In Surviving Mold Illness PART 1 we had focused on problems with Shoemaker's Surviving Mold proprietary **Mold Remediation** procedures. They are touted as being designed to specifically provide the best remediation procedures in cases of Mold-Induced Chronic Inflammatory Response Syndrome, but their recommendations are both expensive and do not work. A bad combination.
- These failed proprietary remediation procedures are one of, if not the major reason that so many Mold-Induced Chronic Inflammatory Response Syndrome (CIRS) patients are not getting better with proper medical treatment.
- In Shoemaker's Indoor Environmental Professionals Panel of Surviving Mold Consensus Statement (SMCS) they feature/recommend many off-the-wall procedures such as (in order to help clean the air) fogging after remediation with a proprietary Glycerin/Borax chemical brew that is actually a serious health hazard.
- No wonder people stay sick. Or even get sicker following such proprietary procedures.
- SMCS has never been Peer Reviewed.
- SMCS has only been reviewed by Surviving Mold Panel Members.

We explained that, with all due respect, we have submitted this 3 Part review to not only Shoemaker but all the co-authors on his Surviving Mold Consensus Statement (2016) and asked for comments/ criticisms.

None were forthcoming except "we got it all wrong".

Hopefully our highly critical review is a wakeup call to fix the glaring errors in SMCS; and will result in a new, improved updated SCMS (Surviving Mold Consensus Statement) that will be put out for Public Peer Review and not only reviewed by his small group of mold assessor adherents/ acolytes.

Shoemaker Recommends Fogging With Hazardous Borax

ThermoFisher SCIENTIFIC

BORAX SAFETY DATA SHEET

Creation Date 16-Nov-2010 **Revision Date** 11-Apr-2016 **Revision Number** 6

1. Identification

Product Name Sodium tetraborate decahydrate

Cat No. : B80; B175500; S24612; S246212; S246250LB; S246500; S249500; S249500LC; NC9821542

CAS-No 1303-96-4
Synonyms Sodium borate decahydrate; Borax

Recommended Use Laboratory chemicals.
Uses advised against Food, drug, pesticide or biocidal product use

Details of the supplier of the safety data sheet

Company
Fisher Scientific
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Emergency Telephone Number
CHEMTREC®, Inside the USA: 800-424-9300
CHEMTREC®, Outside the USA: 001-703-527-3887

2. Hazard(s) identification

Classification
This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Serious Eye Damage/Eye Irritation	Category 2
Reproductive Toxicity	Category 1B

Label Elements

Signal Word
Danger

Hazard Statements
Causes serious eye irritation
May damage fertility. May damage the unborn child

Surviving Mold Illness PART 2 RECAP

- Surviving Mold Illness PART 2: We focused on Shoemaker's Surviving Mold proprietary HERTSMI-2 **Mold Assessment** procedure.
- The proprietary HERTSMI-2 mold assessment procedure is:
  - Not in any way applicable or effective for either Initial Mold Assessment—finding mold that is causing a significant exposure to mold toxins that is often if not usually hidden inside the AC and Ducting (HVAC).
  - Not in any way applicable or effective for Post Remediation Verification determining mold remediation success. Cannot determine if all sources of significant mold exposure have been eliminated.
  - Does not detect any of the other, possibly as/or more important, inflammagens in homes that we collectively call MMIs (Moisture-Induced Microbial Inflammagens) that typically are living in and released from dirty, moist HVAC systems.
- As a result, CIRS patients do not get better even with proper medical treatment.

Surviving Mold Illness PART 3

- Now in PART 3, we show Case Studies highlighting procedures for assessment/remediation that are not only proven to work, but also are relatively inexpensive compared to those featured by Shoemaker's Surviving Mold.
- PART 3 features widely-accepted, straight-forward and again relatively inexpensive procedures to both identify (assess) as well as fix (remediate) ALL significant indoor mold as well as ALL other Significant MMI exposure problems... that are usually in part or in whole related to dirty/microbial contaminated AC and ducting ...
- Even for people highly sensitive to mold, those with Chronic Inflammatory Response Syndrome (CIRS.)
- **Remediation procedures used/recommended are always 100% green, chemical-free.**

FREE Consulting A $75 Value For Only The Cost of This E-Book

- With the purchase of this E-Book you get 15 minutes of FREE telephone consulting to:
  - Answer questions about the material in this Book.
  - Provide second opinion on remediation plans and lab results interpretation.
  - Explain how to perform your own testing.
  - Perform virtual inspections to assess property for microbial contamination and answer general questions about all parts of the investigation and remediation process.

FREE Consulting

- **For SE Florida / Europe:**
  - Contact Linda Rosen (linda@mold-free.org) to set up telephone consulting with Dr. Rosen, book author.
- **For the rest of the US and other international:**
  - Contact Scott Armour at www.armourappliedscience.com

INTRODUCTION

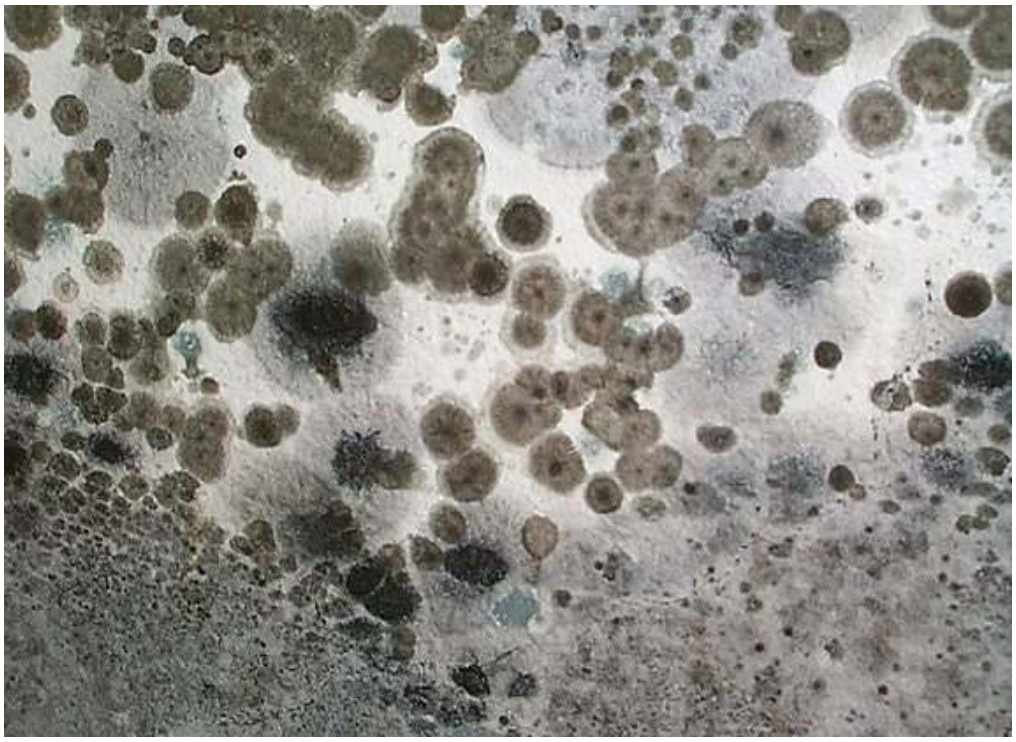
Is It Even Mold That Is The Problem?

- While the focus in the CIRS community has been on illness from mold exposure, rarely are mold spore exposure levels in homes higher than in the outside air.
- Yet, sensitive people are sick in homes from so-called mold toxin exposure but not outside. How does that make sense?
- This is called a Conundrum—a confusing and difficult problem or question.

- It makes sense only in that it is either not mold spore/mold toxin exposure or not mold /mold toxin exposure alone that is the cause of sick homes and sick people.
- And Dr. Richie Shoemaker agrees. He explains that the cause of CIRS in Water Damaged Buildings is a toxic brew of mold, mold fragments, bacteria, viruses (and we add dust mites) that together even at very low levels is the cause of the Environmental Illness commonly called Mold- Related CIRS but may not be caused by mold.
- Could be caused by any of his listed Inflammagens **or more likely a combination or mold, mold fragments and the listed Inflammagens.**

Range of toxins, inflammagens, and microbes found in WDBs		
Mycotoxins	Gram-negative bacteria	Hemolysins
bioaerosols	Gram-positive bacteria	Proteinases
Cell fragments	Actinomycetes	Chitinases
cell wall components	Nocardia	Siderophores
Hyphal framents	Mycobacteria	Microbial VOCs
Conidia	Protozoa	Building material VOCs
Beta Glucans	Chalamydia	Coarse particulates
Mannans	Mycoplasma	Fine particulates
Spirocyclic drimanes	Endotoxins	Ultafine particulates
Inorganic xenobiotics	Lipopoysaccharides	Nano-sized particulates

- Most are microbial in nature. We call this toxic brew Moisture-Induced Microbial Inflammagens (MMIs for short.)
- Table excerpted from Dr. Richie Shoemaker SMCS.



Cause of Mold and MMI Proliferation is Always Moisture

- What Mold/MMIs all have in common … is that they are:
  - Microbial in nature;
  - Require a food source which is moist organic matter (dust, skin cells, animal dander etc.)
  - Require excess moisture (water or elevated humidity) to thrive;
- Of course, testing for mold using even the most sophisticated types of mold testing based on DNA technology cannot detect any MMIs except for mold.
- **Mold DNA testing methodology can only detect at best a small fraction of the inflammagens that cause or aggravate CIRS.**

Infrared camera for detecting surface moisture.

Goal of Inspection

- **The goal of all inspections/assessments** is to find pockets of moisture that will always not only support mold growth but also the proliferation of other Moisture-Induced Microbial Inflammagens (MMIs.).
- Once identified, one determines if these pockets result in significant exposure.
- If so, target for remediation after fixing the moisture source.
- **Once the water source is fixed and the water damaged materials containing microbial growth are cleaned to as new or replaced with new, ALL exposure to MMIs including mold, mites, viruses, bacteria will be eliminated.**
- According to the US EPA recommendations on Mold.
  - Find the moisture, find the mold.
  - Intrusive inspections may be required.
  - Testing/sampling is typically not necessary.

    - Resources are best spent on fixing the mold problems rather than characterizing the mold by testing.
    - Once the mold is gone, what kind of mold was there before remediation is not important.
- And this is good advice. And outside of the specialized world of Shoemaker's Surviving Mold trained mold "experts" that is how it is done.
- A professional mold assessor or home inspector using moisture meters and infrared cameras **looks** for active leaks. Find the leaks or earlier leaks. Find the (Mold/MMIs).
- This will often require follow up intrusive visual inspections for hidden microbial growth (Mold/MMIs) and its cause which is always excess moisture/humidity.
- But Mold Assessors/Indoor Environmental Professionals (IEP) don't generally do intrusive inspections.
- IEPs working with CIRS clients generally **overly rely on surface dust testing (such as HERTSMI-2)** that can never determine the location, extent and cause of the moisture and hence the microbial growth.
- Keep in mind that surface dust testing for mold is said to be a measure of a history of water damage. But it is also a history of how well surfaces are cleaned. If there is mold in dust, clean the dust and there will be no mold in dust.
- And such surface dust testing always misses the HVAC System which is always damp and dirty and usually the most significant source of mold and MMI exposure.
- Identifying mold and MMI exposure requires a professional investigation that includes:
    - Not only moisture measurements and looking for earlier water stains.
    - But also peeking behind baseboards, cabinet toe kicks, dishwashers, and other hidden areas.
    - And visually inspecting inside the HVAC system … carefully checking the always wet/damp AC coils, blowers, housing, AC closets, and ducting for contaminant build up which is always a source of MOLD/MMI growth and exposure.

- Requires intrusive inspection.
- That often means a team approach of specialists under the direction of an Indoor Environmental Professional (IEP) that would include an AC contractor as well as remediators/technicians to perform the intrusive inspection ...and can be a considerable expense.
- But what you spend on a proper assessment you save in money potentially wasted on expensive remediation that does not work.

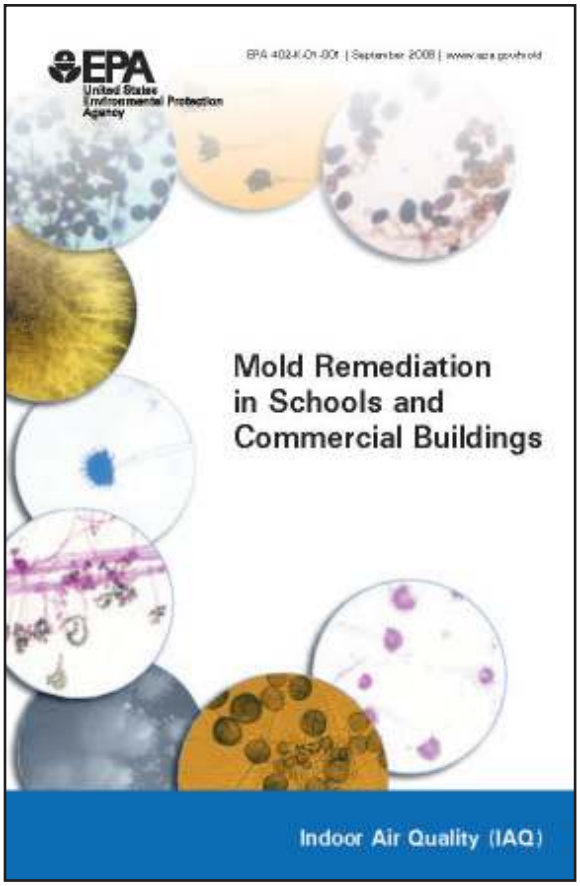
Parts 3A & 3B

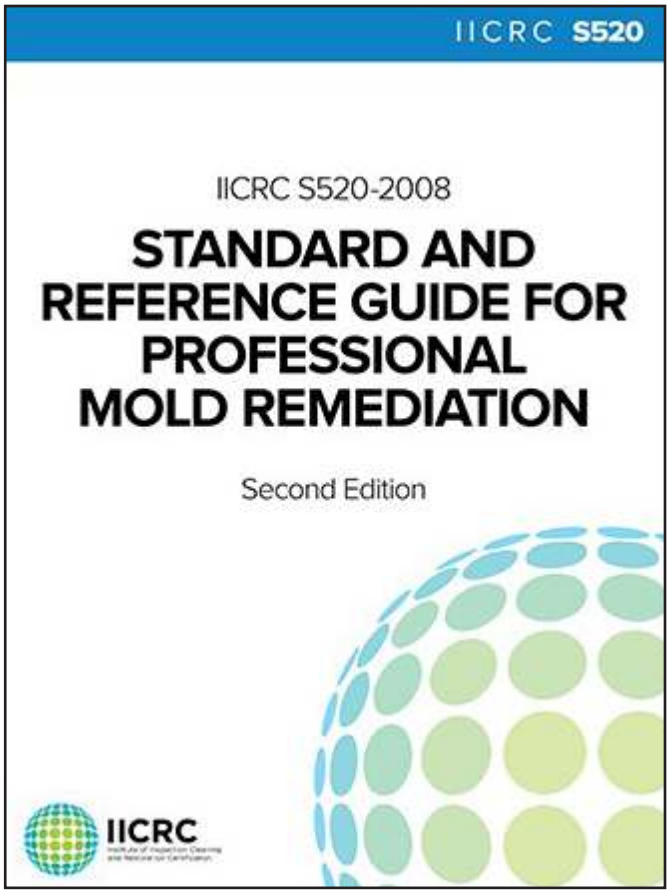
- Part 3A: We explain common Mold Assessment Techniques used in the Case Studies.You need this background in order to follow the Case Study Mold Assessment techniques that show what works and what does not.
- Part 3B: Mold Assessment Case Studies where we have used standard/available:
    - Initial mold assessment techniques to properly identify mold problems for the purpose of remediation.
    - And as important, rule out areas that may be or may have been water damaged (such as sealed walls, attics or crawl spaces) but do not result in significant exposure which is from breathing mold and MMIs.
    - Post Remediation mold assessment techniques to prove that remediation has been effective and that there is no longer significant exposure to not only airborne mold/mold toxins but no longer significant exposure to ANY MMIs.
- If that sounds like a tall order … read on. Hint: We are not using Shoemaker's HERTSMI-2 mold testing procedures for either Initial or Post Remediation Assessment as it does not measure exposure. It tests only for mold (5-species of mold) in settled dust.
- **Our Case Studies will prove that testing surface dust for settled mold spores has absolutely nothing to do with airborne mold (exposure) or other MMI exposure.**

TESTING/ASSESSMENT REQUIRES A TEAM APPROACH

When I Say Testing/Assessment Methods That We Use

- For our firm: Find the moisture, find the mold. Initial sampling/testing is usually not required.
- For our firm: Intrusive inspections are almost always required.
- When I say testing/assessment methods that we use, this is not something proprietary.
- This is right out of the EPA Mold Guidelines and **ANSI-Approved IICRC S500-2015** Standard and Reference Guide for Professional Water Damage Restoration.
- Common sense assessment procedures that work.





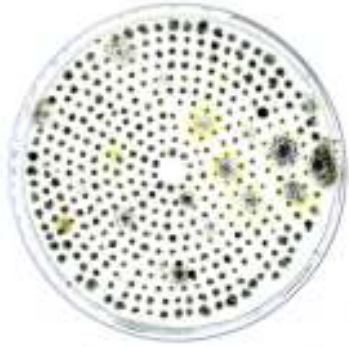
Common Sense Procedures That Work and Are Peer Reviewed

- ANSI-Approved IICRC S500-2015, common sense, practical assessment procedures that work.
- Peer Reviewed and more. Actually ANSI-Approved.
- Approved as an Industry Standard by the American National Standards Institute.

- **Per IICRC S500: "Open components/assemblies as required to determine the source/origin, as well as the full extent of hidden water damage."**
- **No false positives or false negatives. Seeing is believing. That's our philosophy. That's what works.**

Finding All Hidden Mold and All Sources of MMI Exposure

- Finding all hidden mold/sources of significant exposure requires a Team Approach.
  - Indoor Environmental Professional (IEP)/ Mold Assessor.
  - Remediation Technician to set up environmental controls for intrusive inspections.
  - AC contractor for full visual access and assessment of the interior of the HVAC System including ducting.



TESTING/ASSESSMENT PHILOSOPHY

When Do We Test?

- We always take post remediation air samples to make sure that we have not cross-contaminated the indoor environment during mold remediation.
- Generally we do not perform any initial mold testing. We rely on visual assessment for mold and current or previous moisture sources, aided by moisture detection equipment such as moisture meters and infrared (thermographic) cameras.
- Resources are best spent removing mold rather than characterizing mold.
- Once the mold is remediated/removed, what type of mold was there before the remediation is not important.
- Find the moisture, find the mold and as the HVAC System interior is always moist and always dirty/dusty (food source for microbial contaminants), always focus on the HVAC System when you are concerned about exposure for CIRS.
- Focus on visual inspection. Seeing is believing.
- Look for sources of significant exposure which means airborne inflammagens.
- Rule out hidden mold that is not a source of exposure... airborne inflammagens.
- Often (always for CIRS clients) requires intrusive inspections. Inspecting behind baseboards, under carpet, behind dishwashers, etc. And thoroughly inspect the AC and ducting.

Infrared camera.

Moisture meter.

Inspection Goal. Not Only Finding Problems ...

- Inspection goal. Not only finding areas of concern, but also ...
- Determining what areas of concern represent exposure or potential exposure and what do not.
- **We fix what we need to fix to eliminate exposure.**
- **We do not fix (we rule out) areas of potential concern that do not represent exposure.**
- **The cost savings of fixing only what causes exposure, pays for a thorough Team-based assessment/ inspection many times over.**

Goal is Always to Provide an Irritation-Free Warranty

- **If the home owner says they are sick in the home but fine outside, that is all the "testing" we normally need.**
- **Find the source of significant exposure and restore to "as new".**
- **Restoring to "as new" eliminates not only all significant mold exposure problems but all significant MMI exposure.**
- **At that point we can always provide a Guarantee/Mold & Irritation Free Warranty for our work which is that there is no longer detectable illness or irritation specific to the home.**
- **We say generally we do not do pre-remediation sampling/testing. But for the following three Case Studies we do extensive testing. These are special cases.**
- **But again: The cost savings of fixing only what causes exposure, pays for a thorough Team-based assessment/ inspection even with extensive testing ... many times over.**

TESTING METHODS

Summary of Mold Testing Methods That We Used For Our Case Studies

- **Testing methods used:**
  - Spore Trap Sampling (Dead+Viable Spores) analyzed by Direct Microscopic Examination (DME).
  - Laser Particle Counting of the Air
  - Dust Sampling by DME
  - Visual Surface Testing by Swiffer
  - Air Sampling for Viable (New/Live) Spores
  - Dust Sampling by DNA methods
  - Air Sampling by DNA methods
- There is no single testing approach that comes close to meeting all needs for either finding mold or ruling out mold exposure.
- Different testing procedures test for different things. And generally complement one another. Answering different questions.
- For instance, viable air sampling tests only for live/viable spores.
- Spore traps test for total spores (both dead and live spores)
- As spores have limited life expectancies, testing for the ratio of airborne viable/new versus spore trap total spores ...
- Gives you an indication if there is a current problem or sampled spores are mostly old/dead and likely from the outside air which consists of mostly old/dead spores.
- A high ratio of viable to total means new fresh mold.
- Inspections/testing can get expensive, but no matter how expensive, testing always costs much less than failed remediation.
- To keep the cost of inspections low, focus on visual/moisture inspection and less on testing.
- As mentioned, we generally perform no initial sampling.

- However, while both the EPA and IICRC agree that initial testing is often not required, clients and/or their doctors often consider (wrongly) that the key to assessment is testing.
- Therefore one must test if that is what the client expects… and they often do.
- Our typical procedure if someone calls us for testing because they believe the home has problems, but they don't know where, is to recommend that they have the home inspected / tested by licensed home inspector that is also a licensed mold assessor and send us the report. We want to see lots of high quality pictures.
- Such inspections are based on/ focus on visual assessment for mold and moisture with the aid of moisture detection equipment. And include a few spore trap air samples.
- Once we see the report, we can then decide on what type of team is required to perform the follow-on inspection (if any) needed by us to provide a quote to repair/ remediate.
- Some clients are concerned about one party performing all aspects of the job including initial testing and preparing the protocol for work because it has been explained to them that this is a conflict of interest.
- But often the mold assessor is far less qualified than the remediator.
- In Florida one can get either a Mold Assessor or Mold Remediator license with 14 hours of online training and a 2-hour multiple choice test.
- Most other states have no licensing requirements.

Air sampling pump.

- But even if the Assessor is highly trained you never know until the walls or ceilings are opened what the extent or oftentimes even the origin of the mold growth.
- You never know what you need to know to fully remediate until remediation actually starts.
- So what is the value of a detailed "protocol" written by an independent mold assessor? It has limited value.
- The mold assessor should point out the problems to be remediated and /or problems to be further investigated. How the remediator does his job should be up to the remediator.
- The only mold remediation protocol that we use is:
    - Remediate to the extent needed to restore to "as new" at the locations identified in the inspection report.
    - Procedures are 100% Green, Chemical-Free.
    - We test the air after remediation and rebuild are complete to make sure we have not left the home's indoor air contaminated.
    - We provide a mold free warranty for all work performed. That's easy because everything in the work area is cleaned to "as new" or is new.
- Oftentimes the Assessor fills their report with pages and pages of boilerplate on how to test and how to remediate that is not at all specific for the job at hand.
- This is to look like you paid money for an extensive and useful report.

- For CIRS patients, you only want the Assessor's opinion on 5 things:

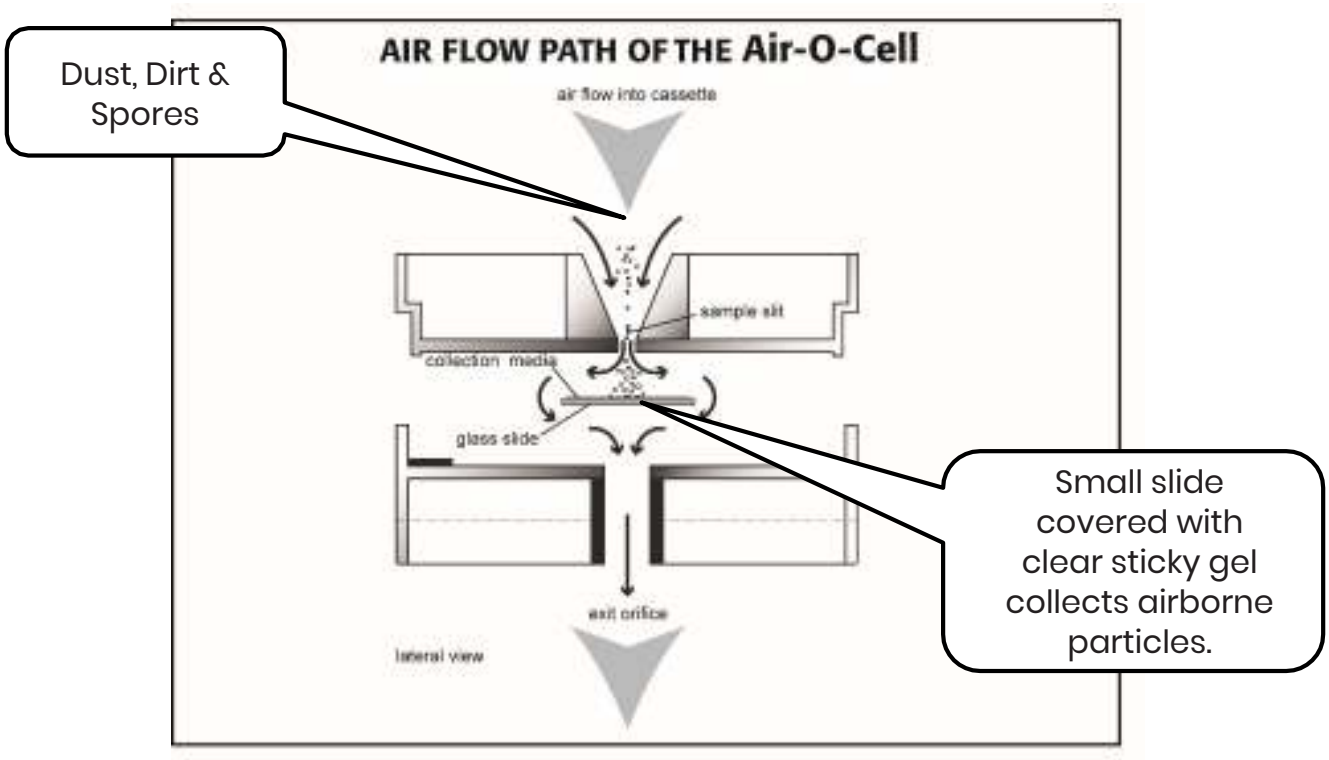
    1. Are there any active leaks?
    2. The location of new or earlier mold problems or possible problems for the purpose of getting mold remediation quotes.
    3. And just as important for CIRS patients, you also want to know which of these problem areas is likely to cause significant exposure. Save your money. Hidden mold inside or walls or ceilings from earlier leaks that does not result in exposure should not be remediated.
    4. And if hidden mold inside of walls or ceilings can result in exposure, can properly sealing any openings around lights, registers or holes be enough to eliminate exposure risks or does the mold inside need to be remediated /removed.
    5. **If the Remediator performs the items listed to be fixed, will the Assessor provide a guarantee/warranty? If not, what needs to be done for the Assessor to so provide?**

Stachybotrys Toxic Mold

SPORE TRAP AIR TESTING

Spore Trap Air Sampling

- Mold assessors test for "total" airborne mold spores (live and dead) using spore traps (air sampling cassettes).
- Spore trap sampling collects mold spores from the air onto small sticky slides inside the air sampling cassette.
- This is the traditional method and still the most common method of mold air testing.
- It detects all species of mold spores in the air. It is not limited to only 5 species as with Shoemaker's HERTSMI-2 or 36 with EPA's ERMI.



- After the air samples are delivered to the lab, the micro slides are removed from the cassettes at the lab are analyzed by **Direct Microscopic Examination (DME).**
- Cannot distinguish dead from live spores with DME.
- Cannot distinguish small (respirable) spores in the 2-3 micron range from one another, which are grouped together and called Penicillium/ Aspergillus-Like.
- Spore traps cannot detect anything smaller than a spore …cannot detect sub-micron mold fragments that are below the detection limit of Direct Microscopic Examination.

Spore Trap Limitations Regarding Fragments. A Huge Issue.

- The latest science has found that mold fragments are much more abundant than spores and are more inflammatory/toxic than the much larger spores.
- Fragments are not detected by spore traps as they are below the detection limit of DME.
- However, spore traps are 1/10th the cost of an ERMI DNA air sample with same day turn around, versus 4-5 days for DNA, and remain quite popular with mold assessors.
- We use traps to supplement ERMI air sampling.
- But again, we generally do not perform any initial mold testing but rely on intrusive visual inspections and moisture detection equipment to find potential exposure problems for the purpose of determining what and where to remediate.

- On the other hand, we always take post remediation air samples (spore traps) to make sure that we have not cross-contaminated the indoor environment during mold remediation.
- Only rarely for special situations do we take expensive post remediation DNA air (or dust) samples as we do in these Case Studies.

LASER PARTICLE AIR TESTING

To Improve The Sensitivity Of Spore Trap Testing ....

- Dust interferers with DME (Direct Microscopic Examination). If there is significant dust in the air being sampled, taking longer duration air samples will actually reduce the measured spore count per volume of air because the collected spores become obscured by dusts.
- For that reason, the duration of spore trap sampling is typically limited to 3-5 minutes unless one can determine that the indoor environment being sampled is dust free or relatively dust free.
- If the air is dust free or relatively dust free, you can take longer air samples and greatly improve collection accuracy.

Before Taking Spore Traps

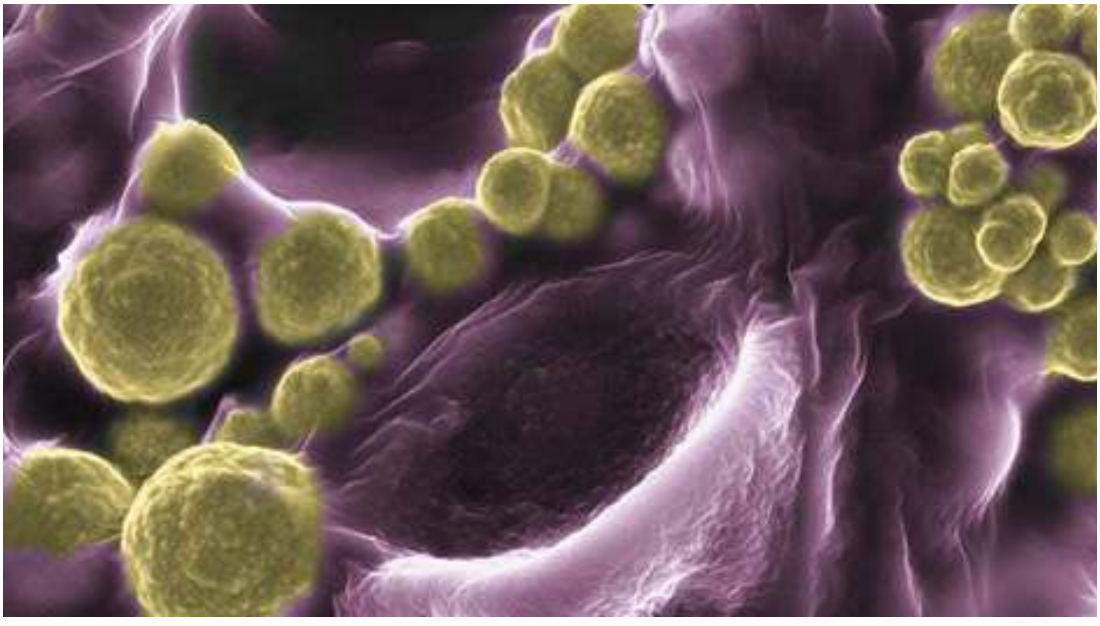
- Before taking spore trap air samples, we check the levels of particles in the indoor air with a laser particle counter.
- And compare to a known clean standard.
- If relatively dust free, run the sampling pump for 10-20 minutes rather than 3-5 minutes.
- This will greatly improve the sensitivity of the spore sampling .... but still will not measure mold fragments of course whose size is below the detection limit of DME.

Laser particle counter.

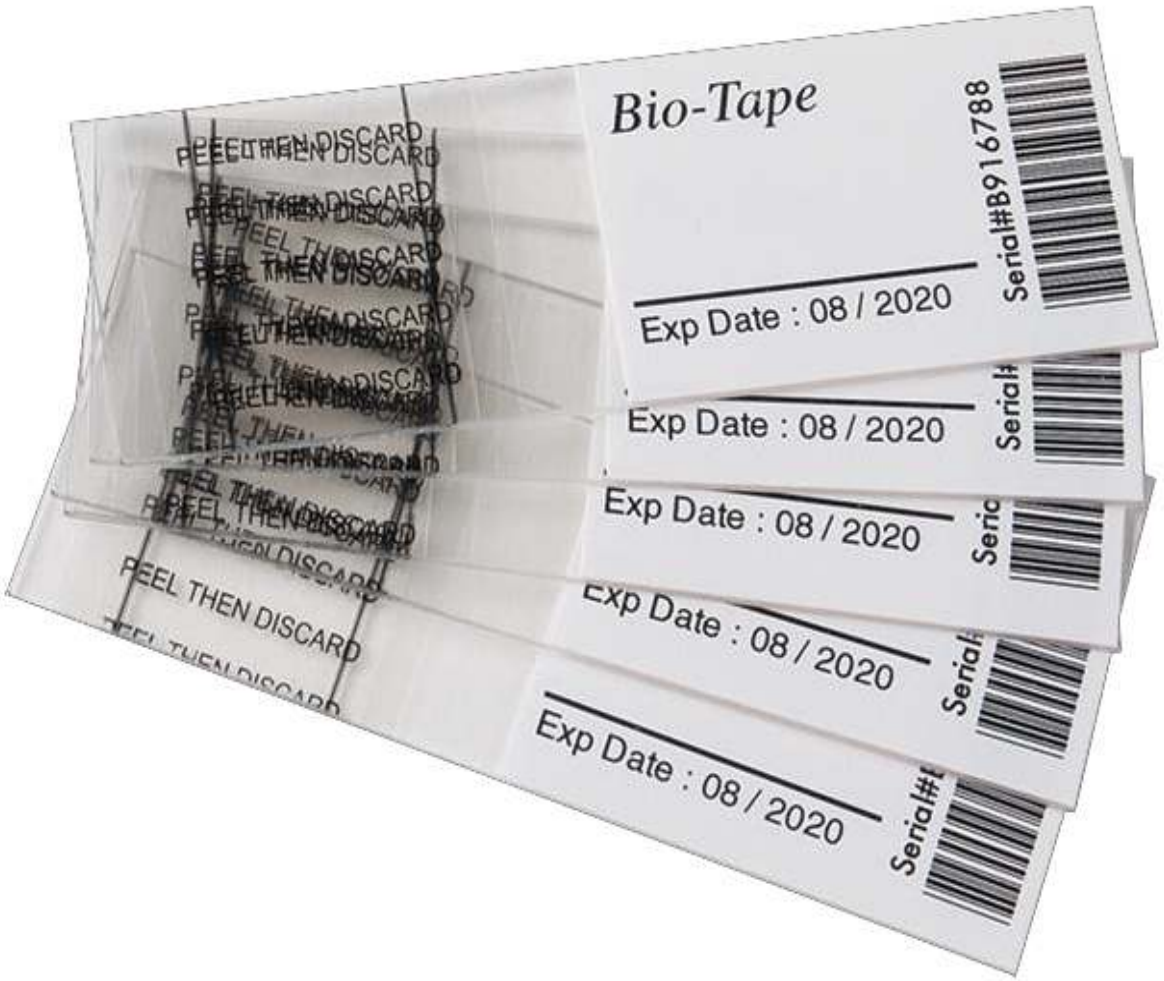
Example Laser Particle Counter Results

	1.0 micron	2.5 micron	5 micron	10 micron
Elevated dust	9.4K	2.5K	.7K	.4K
Clean control	2.4K	.1K	.06K	.05K

- Numbers are in particles per cubic meter of air.
- Longer and more accurate spore trap sampling times are appropriate in super clean environments.



DUST TESTING BY DME



Surface Dust Analyzed by DME

- Surface dust sampling for total (dead + live) spores analyzed by DME.
- Lift tapes, flexible microscope slides, and swabs.
- Or bulk samples of drywall in a zip lock bag.

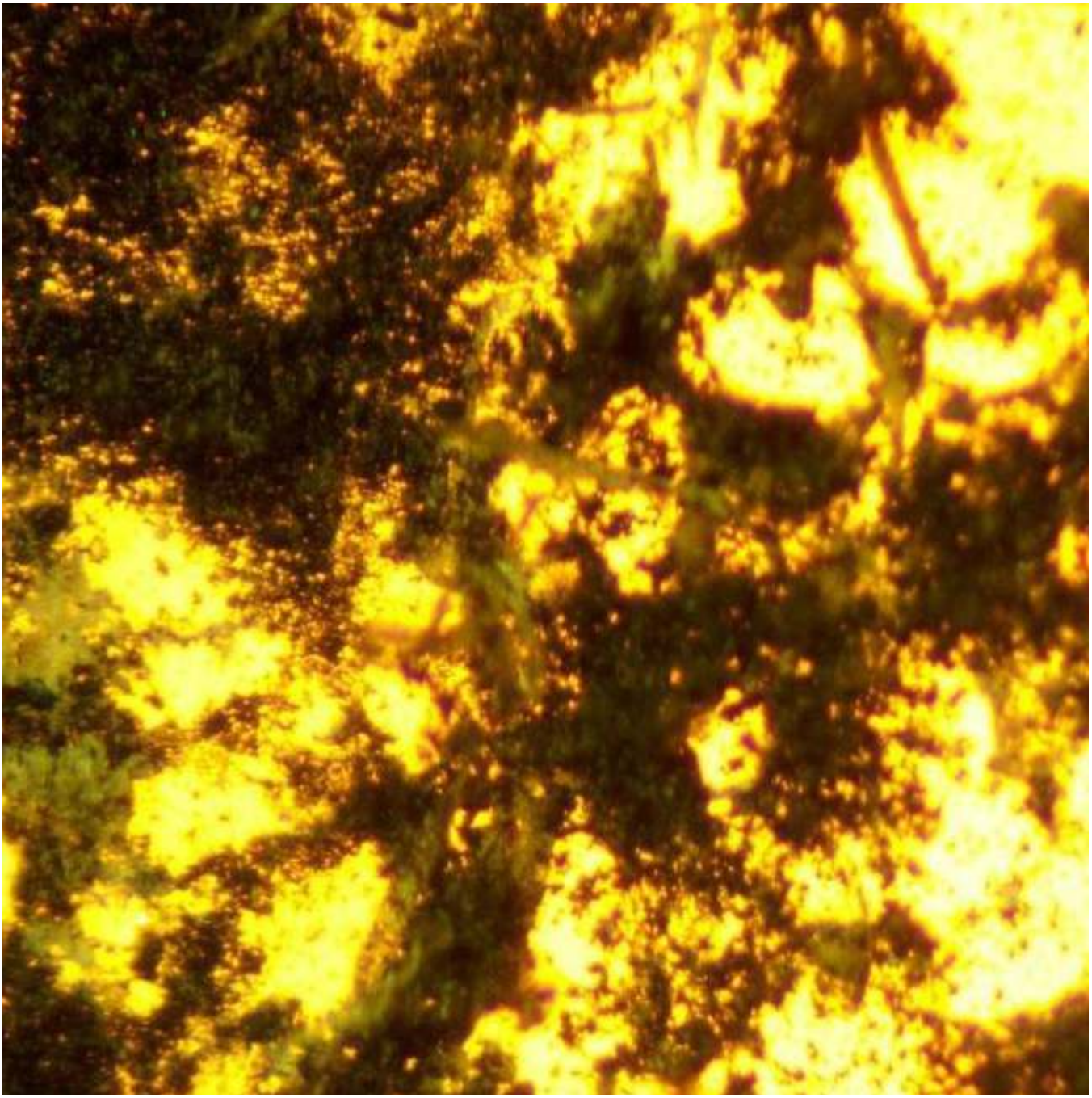
Flexible slide for taking surface samples (called lift tapes).

Surface Dust Sampling by Swab, Slide/Tape or Bulk

- No method of surface dust sampling by Direct Microscopic Examination (DME) is quantitative:
  - Surface dust interferes with the analysis/ determination of the number and type of spores.
  - Swab, tape or bulk sample collection methods do not result in consistent size samples.
- Most professional mold assessors should find this method of mold testing of little to no value. But they commonly take lift tapes or swabs because clients expect them.

Surface Dust Sampling by Swab, Slide/Tape or Bulk

- However photomicrographs of lift tapes do look pretty cool and can spruce up a report. Below is a 40x magnification of Stachybotrys (commonly called the Black Mold). Looks ominous.



SURFACE TESTING FOR CLEANLINESS BY WHITE GLOVE TEST

White Glove Dust Post Remediation Cleanliness Test With Swiffer

- Clean surfaces with Swiffer. (Swiffer contains alcohol, detergent and antimicrobials.)
- And then "re-clean" areas. Check for dirt on the Swiffer.
- We call this "White Glove" testing.

Swiffer (White Glove) For Surface "Testing"

- Use White Glove testing (we use a wet Swiffer) to visually check for the absence of settled dust either on surfaces or inside AC ducting.
- No settled dust = No mold spores/fragments in the settled dust.
- How is this determined "scientifically"?

"Testing" Settled Dust EPA Procedure

- All mold assessors are required to take the EPA Lead Paint assessment class.
- Each student receives a laminated card that allows them to determine what the EPA calls Elevated dust or Not Elevated.
- While developed for lead paint dust, this is appropriate for mold work as well.

Swiffer Test of Floor Dust

- Wipe the surface (floor or inside ducting) with a wet Swiffer.
- And compare to pix at bottom.
- If darker, this is defined as elevated dust. FAIL.
- If lighter … PASS.
- In our experience the Swiffer test is by far the best method for checking the settled dust for spores/fragments after mold remediation …
- For checking the level of spores in dust. How's that?You might say "it does no such thing." But…
- No dust = No spores/fragments in the dust. And that's what we want to know.
- If there is dust/discolored Swiffer … continue cleaning until there is no dust and therefore no spores/fragments in the dust.

- Swiffer white glove testing for (ruling out) mold spores/fragments in surface dust.
  - No dust. No spores in the dust.
  - No waiting for lab results.
  - Seeing is believing.
  - No possibility for error.
- We like. We use.

AIR SAMPLING FOR VIABLE SPORES

Bioaerosol Impactor is a sampler for collecting viable mold and fungi

Viable Air Sampling Benefits

- The 1st benefit of viable air sampling vs spore traps includes the ability to distinguish species in addition to genus which is required to determine if spores are water damage indicators or simply background (outside) spores.
- The 2nd benefit is that viable air sampling only counts newer live/viable spores and not old/dead spores (vs spore trapes) and gives a better indication of recent water events.
- The 3rd benefit is that culture sampling is more effective in capturing small respirable mold spores (Pen/Asp) than spore trap sampling.
    - But of course will not detect mold fragments which are not viable/ will not germinate. Only intact spores will germinate.
- The 4th benefit is that viable testing is less affected by dust in the air than spore traps.
- Lastly, it is much less expensive than DNA-based test methods. Similar in price to spore traps at least with the lab we use (ProLab).
- Yet rarely is viable mold sampling used by mold assessors.
- Why?
    - Takes time to get results back due to incubation.
    - Special equipment needed.
    - Need to refrigerate media.
- Even though better than spore traps, rarely used by most mold professionals.
- Viable air sampling.... might be a little bit of a pain compared to spore trap air sampling.
- But we like it.
- We use it in addition to spore traps.

Viable Air Sampling Pros & Cons

- If someone is sick, why not use the best testing options available and use multiple testing methods that complement one another … but always keeping in mind this is to supplement visual assessment/moisture testing.
- Always keeping in mind that as with spore traps, viable air testing does not detect mold fragments that are typically the major cause of mold induced CIRS.
- Only DNA/PCR air testing does.

Viable Sampling Gives a Visible Result

- Nothing spruces up a report like pictures of mold cultures.
- Keep the culture samples for a few days before you send them to the lab for analysis.
- Take a picture before sending them in. This looks way cool.
- **But more importantly gives the client a visual as to the extent of the mold. Seeing is believing.**
- For example, see the following pictures comparing mold outside to inside after remediation.

Outside control. Lots of mold.

Post remediation indoor culture sample. Almost perfect.

DNA/PCR DUST & AIR TESTING

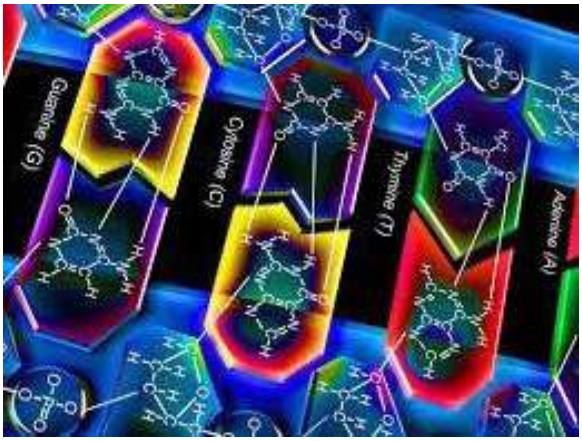
Mycometrics/Shoemaker on ERMI
https://www.mycometrics.com/document/articles/ERMI_Lin_Shoemaker.pdf

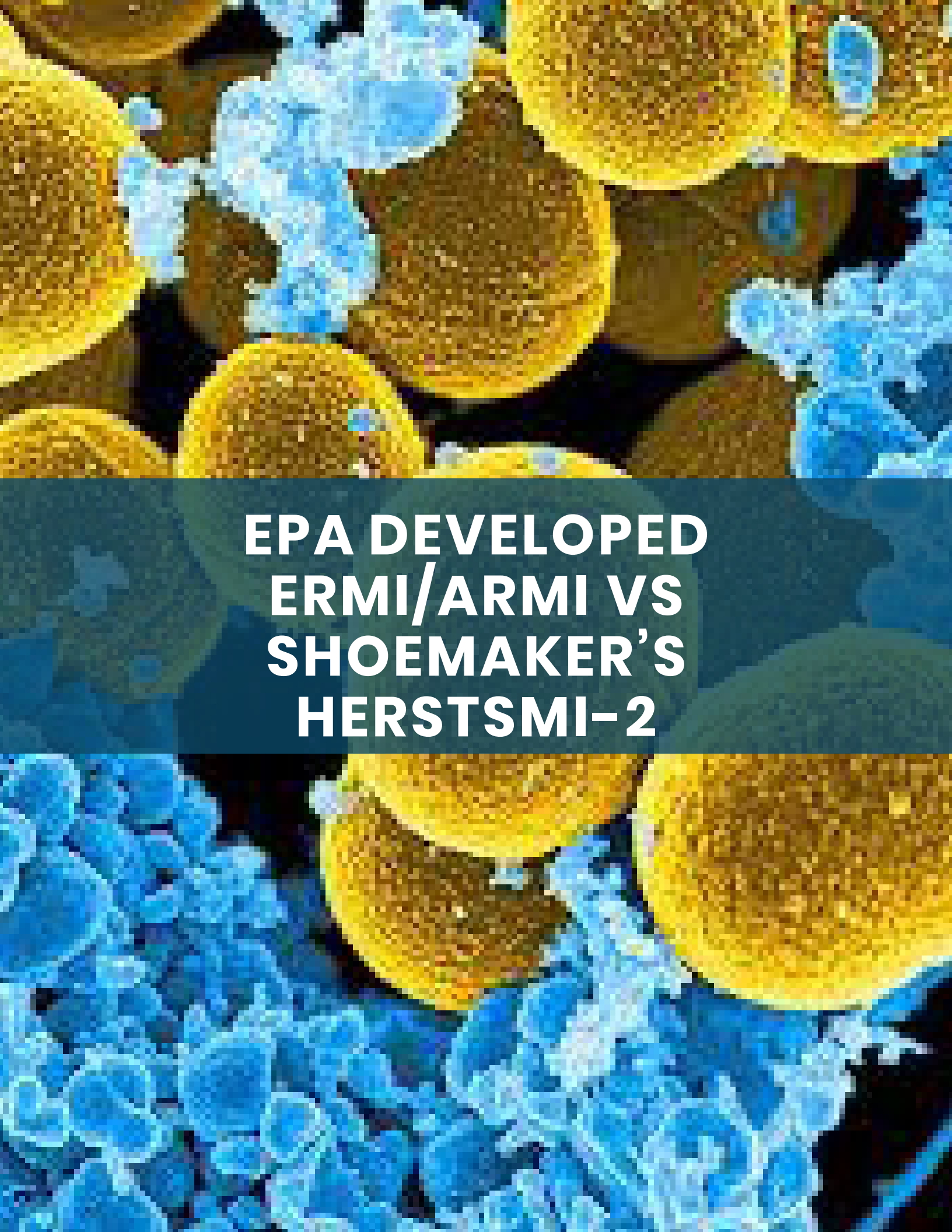
- Back in 2007, Drs. Lin and Shoemaker: (Dr. Lin is the Technical Director of Mycometrics) published an important seminal article reviewing a new technique developed by the EPA for measuring mold, based on DNA analysis.
- The article praises/highlights the many benefits that DNA sampling has over traditional (non-DNA) mold testing methods.

- One of the breakthroughs of DNA mold sampling is that DNA sampling detects mold fragments in addition to spores.
- Mold fragments are invisible to traditional testing but are more numerous and more of a health problem to mold sensitive people than spores.
- Therefore DNA testing that counts fragments in addition to spores was a game changer.
- Lin/Shoemaker in the article explain that DNA analysis for mold/fragments can be used for both air sampling and dust sampling.
- Lin/Shoemaker extol the many virtues of DNA air sampling compared to air sampling with traditional spore traps …
- But then their sole focus moves to DNA dust sampling.
- Not air sampling. No explanation is given.

Mycometrics/ Shoemaker Focus Moves to DUST

- Our assumption is that the reason the sole focus of Lin/Mycometrics and Shoemaker is DNA dust sampling and not air sampling is that dust sampling is very simple/ very easy.
- Simple. Can be done by a consumer with no special training and no special air sampling equipment.
- But simpler is not necessarily better.
- Mold in pockets of dust does not measure actual exposure. In fact, DNA testing for mold always greatly over-estimates mold in the air that is what represents exposure.
- Many mold assessors use the very high values of mold DNA in dust as a scare tactic to push expensive remediation.
    - Finding mold in dust in no way helps determine if there is significant mold exposure.
    - Finding mold in dust in no way helps determine the source of exposure for the purpose of remediation.
- If there is mold in the settled house dust … clean the dust. Then no mold in the dust. This is not Mold Remediation. Its cleaning.
- Focusing on DNA dust sampling instead of DNA air sampling … In the following case studies we show that this is just plain wrong.
- Dust sample analysis by DNA does not measure actual exposure which is always from breathing mold + fragments and not from mold in pockets of surface dust.
- **Again, if there is mold in the dust. Clean the dust. Then no mold in dust.**




EPA DEVELOPED
ERMI/ARMI VS
SHOEMAKER'S
HERSTSMI-2

ERMI/ARMI vs HERTSMI-2

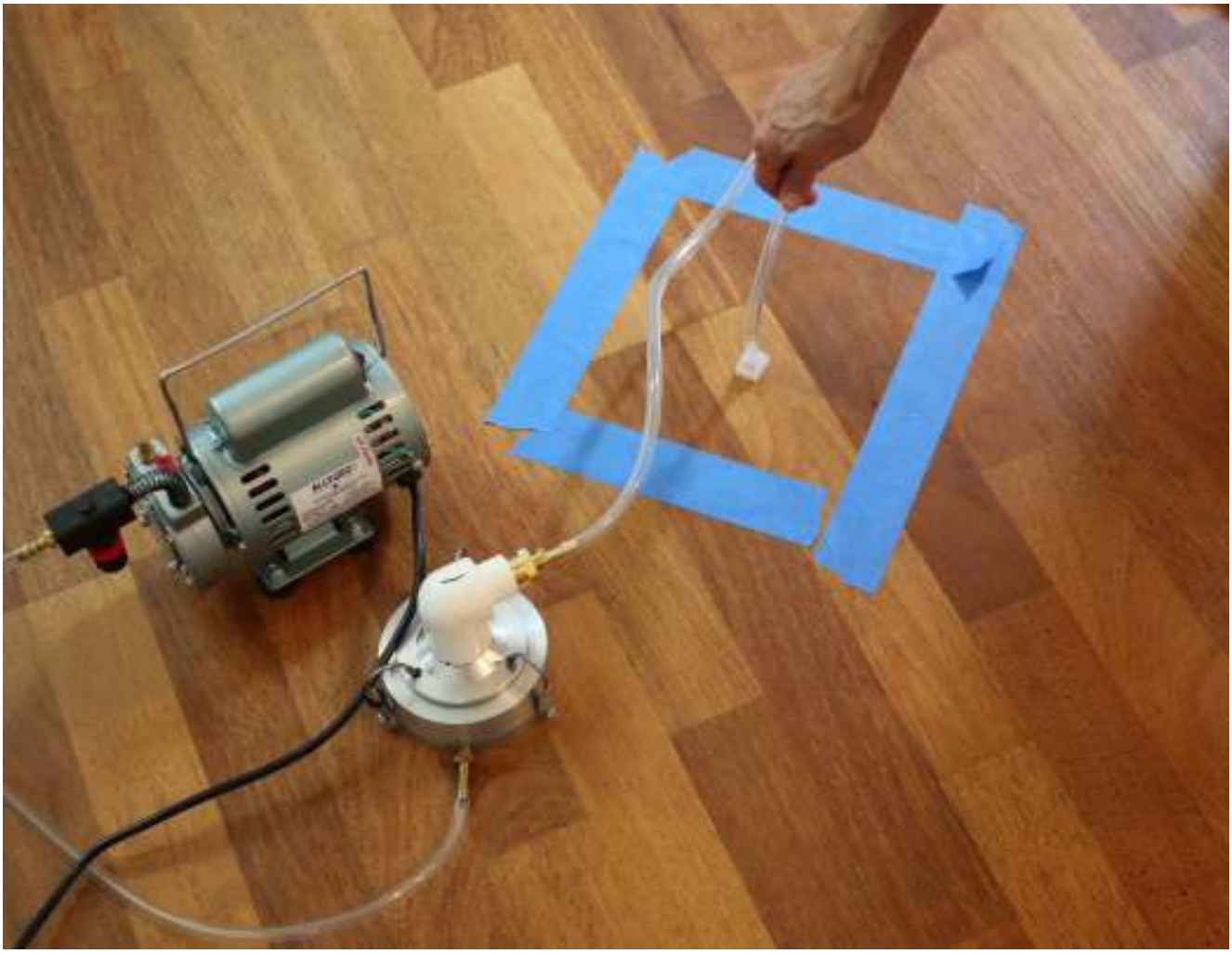
- We have never performed any testing with Shoemaker's HERTSMI-2.
- We perform DNA testing only with EPA developed ERMI or ARMI.
- ARMI (American Relative Moldiness Index) is an EPA developed subset of ERMI (13 vs 36 species.)
- According to the EPA, ARMI provides almost the same amount of information (about 80%) as ERMI for **about half the price.**
- If your client wants to save money or have the budget to take multiple samples, the ARMI is about the same price as HERTSMI-2 with 13 molds tested (vs 5 for HERTSMI-2.)
- And ARMI is industry standard. Developed by the US EPA that invented the DNA testing technology.
- Since ARMI is a superset of HERTSMI-2, if you would like a HERTSMI-2 reading, ask your lab to also provide the HERTSMI-2 reading for the ARMI test.
- There is never a reason to use HERTSMI-2.
- And ARMI (just like ERMI, but unlike HERTSMI-2) is designed for both air sampling as well as surface dust sampling.
- I can't say this enough … testing isolated pockets of dust for mold with HERTSMI-2 does not give you any indication of exposure which is from breathing mold.
- Only sampling the air gives you a measure of actual/ current exposure to mold, mold fragments and mold toxins.

AC supply plenum full of toxic mold.

Measuring Mold In Dust Always a Waste of Time & $$

- And once more, if there is mold in dust it never needs to be measured/ characterized.
- Clean the dust.
- After cleaning, test the surfaces with a Swiffer to prove there is no longer surface dust.
- And when there is no dust there is no mold in the dust.
- **That is all we want to know about dust.**

Collecting surface dust for analysis.



THREE CASE STUDIES

Case Studies Follow

- In the 3 Case Studies that follow we compare test results for:
  - Clean the dust.
  - Spore Trap (Air sampling)
  - Culture/Viable (Air sampling)
  - DNA Air and Dust Sampling
- We will clearly show the benefits of DNA air testing, that detects mold fragments in addition to spores, for measuring current exposure.
- And we also explain its limitations.

The Goal of Any Inspection

- The goal of all inspections/ assessments is to find pockets of moisture that will always support not only mold growth but also the proliferation of other Moisture-Induced Microbial Inflammagens (MMIs.).
- Once identified, one determines if these pockets result in significant exposure.
- If so, target for remediation after fixing the moisture source.
- Keep in mind that testing — even DNA air testing — should be in conjunction with visual inspection methods by a professional mold assessment team using moisture detection equipment and include intrusive inspections behind dishwashers, baseboards, etc.
- And the assessment should always include intrusive inspection inside of the entire HVAC system when there are persons with CIRS or anyone supersensitive to mold involved.

Infrared camera for moisture detection.

Moisture meter.

Case Study #1: Clean Control

- Case Study #1: My home.
- This is the Clean Control.
- No leaks.
- We tested the heck out of it. It is clean.
- Tests show no measured mold or mold fragment exposure.

Case Study #2: Robert B. Home Clean Control

- Case Study #2: Robert B. home. Type 1 Error. False Positive for mold exposure in his home. There is no mold exposure but in this case his doctor says that there is.
- Home is clean/Mold exposure free but has been incorrectly categorized as a problem/moldy home by his physicians.
- His physicians prescribed urine testing that comes back highly elevated for mold toxins. As a result of these findings his physician claims his home must be contaminated. Find the problem. Or move out.

- We agree with Dr. Shoemaker that these proprietary urine tests are not reliable. See his important paper on in his SurvivingMold.com web site: Urinary Mycotoxins: A Review of Contaminated Buildings and Food in Search of a Biomarker Separating Sick Patients from Controls which concludes:
- **Shoemaker says: "... there is no basis to ascribe any diagnostic significance to urine mycotoxin testing."**
- We agree. See additional analysis of mold toxins in urine at our sister web site: www.Mold-Toxins.com.

Case Study #2: RB Home Type 1 Error.

- RB's ISEAI (www.ISEAI.org) accredited/trained physician heavily relies on urine testing to determine if there is current/continuous mold toxin exposure.
- As Shoemaker has shown in detail, such testing is not reliable. Results in what is called a Type 1 Error. Determining that there must be current mold exposure but there is none.
- As a result of this incorrect/erroneous conclusion, RB is being treated for CIRS-WDB, treated for continuous exposure to mold toxins where no toxin exposure exists.
- Case Study #2 proves the home is mold exposure free. No significant numbers of mold spores or fragments in the air.
- Therefore Case Study #2 confirms that urine testing for mycotoxins that "detects" current high levels of mold toxin exposure is in Error.

Case Study #2: Karen S. home. Type 2 Error

- Karen S. home. Type 2 Error. False Negative. There is mold exposure, but people think there is none.
- She is under treatment for CIRS. This was a newly purchased home with absolutely no content in the home. No carpet.
- Super clean in appearance.
- But Karen immediately got sick whenever she entered the home in a matter of minutes.

- In our experience that is always a 100% accurate test for mold and MMI exposure. Just need to find the source of exposure which is usually AC/ducting related when the home is frequently cleaned of dust and there is no old/dirty carpet or clutter.
- Her husband thought she was a nut case.
- Her mold assessor thought she was a nut case.
- But as we shall see … not at all.
- Mucho mold. But hiding. In this case because it was a resale, mold and water damage problems were not only hiding but evidence was purposely hidden/ covered up by the seller.
- Typical … unfortunately.

After Case Study Review Reader Should Clearly Understand

- After these case study reviews, the reader should clearly understand why testing for mold DNA in pockets of surface dust makes no sense vs DNA air sampling as a measure of mold and mold fragment exposure.
- Why the focus of the mold investigation should always include looking for hidden problems in the HVAC System where even a small problem can represent a large exposure.

After Case Study Review Reader Should Clearly Understand

- After these 3 Case Study reviews, the reader should clearly understand the benefits (requirement) of a Team approach to a complete initial assessment which would include:
  - Indoor Environmental Professional (IEP)
  - Remediator/Technician &
  - AC contractor

A Complete Initial Mold Assessment Will Include:

- A thorough visual inspection for current or prior sources of moisture using moisture measurement tools, and perhaps some limited testing.
- With the aid of a remediation technician using proper containment methods, an intrusive inspection checking behind baseboards, behind dishwasher, inside of drywall, behind cabinets if needed to determine the:
  - Origin/source,
  - Location,
  - Extent
- Of water damage and MOLD/MMIs as sources of exposure for the purpose of determining what and where to remediate.
- With the aid of an AC contractor, checking the inside of the entire HVAC System for cleanliness/ sources of exposure.
- If there is accumulated dust within the AC and ducting, the humidity inside the HVAC system will result in microbial growth on the moist dusts.
- The growth may be any combination mold, bacteria, viruses, and/or dust mites that feed on mold.
- Always beyond the AC filter resulting in exposure.

Post Remediation Verification Inspection

- The Post Remediation Verification (PRV) inspection, by its nature, in order to rule out any current exposure, must rely on testing along with visual inspections for cleanliness.
- **When there is a CIRS patient involved, and we want to rule out even trace levels of mold exposure. PRV testing should be by multiple methods, because as we have pointed out earlier, each particular testing method has its own advantages as well as disadvantages.**
- **They complement one another PRV testing should always include surface dust surface and air testing (spores traps or DNA).**

CLEAN CONTROL (MY HOME) CASE STUDY #1

Before Taking Spore Traps

- There are no active leaks/ moisture or humidity problems in my home (Clean Control.)
- Before taking spore trap air samples I checked the levels of particles in the indoor air with a laser particle counter.
- My home has essentially no fabric furniture. No carpeting. No open windows.
- Floors cleaned 3x per week (due to dog hair.)
- Super high quality Merv 13 air filters in all AC returns.
- AC FAN=On 24/7. Super clean HVAC system.
- We know we have relatively little dust in the air compared to most other homes.

Laser Particle Counter Results

- Numbers are in particles per cubic meter of air.
- Air very clean of particles 1.0 to 10 micron (mold spore size range).
- Because air is very clean, we decided to use spore trap sampling time of 10 minutes instead of typical 3-5'.

	1.0 micron	2.5 micron	5 micron	10 micron
Dr. Rosen Home Case Study #1	2.4K	.1K	.06K	.05K

- Took 2 identical 10 minute spore trap samples and two outside.
- The indoor sample levels are ultra low as expected, I've tested my home many times.
- See lab results next page.

Mold Spore Trap Sampling Clean Control. Very Low. Not Elevated

ANALYSIS METHOD	6110 Air Direct Examination			6110 Air Direct Examination		
LOCATION	INDOOR CLEAN CONTROL			INDOOR CLEAN CONTROL		
COC/LINE #	1257446-1			1257446-2		
SAMPLE TYPE & VOLUME	AIR-O-CELL 100-150L			AIR-O-CELL 100-150L		
SERIAL NUMBER	28374379			28374391		
COLLECTION DATE	July 30, 2019			July 30, 2019		
ANALYSIS DATE	July 31, 2019			July 31, 2019		
CONCLUSION	NOT ELEVATED			NOT ELEVATED		
IDENTIFICATION	Raw count	Spores per	Percent of Toal	Raw count	Spores per	Percent of Toal
Cladosporium						
Curvularla	1	7	33	1	7	50
Nigrospora	1	7	33			
Other Ascospores						
Other Basldospores	1	7	33			
Pencillum Aspergillus				1	7	50
Peronospora						
Pithomyces						
Torula						
TOTAL SPORES	3	21	100	2	14	100
MINIMUM DETECTION LIMIT	1	7		1	7	
BACKGROUND DEBRIS	Light			Light		
Celluslose Fiber	1	7		1	7	
OBSERVATIONS & COMMENTS						

Mold Spore Air Testing Results. My House. Clean Control.

- Also note that "Background Debris" in my home is Light which means clean of dust on the Air-O-Cell micro slide. (Left two columns = Indoor/My Home).
- By comparison right two columns = Outdoor. Background debris is **Moderate.**
- When debris is light, the lab is able to accurately count the spores present. Spores are not covered/obscured by dust particles.

TOTAL SPORES	3	22	100	1	7	33	1	7	33	11	75	100
MINIMUM DETECTION LIMIT	1	7		1	7		1	7		1	7	
BACKGROUND DEBRIS	Light			Light			Moderate			Moderate		
Celluslose Fiber	1	7		1	7							
OBSERVATIONS & COMMENTS							Non bilogical debris present			Non bilogical debris present		

Lift Tape of Surface Dust on Top of My Dining Room Cabinet

- Lift tape from pocket of surface dust on top of cabinet.
- Dusty. Hardly ever cleaned.
- Very low spore count based on Direct Microscopic Examination (DME)

Lift Tape of Surface Dust On The Top of My Cabinet. Nothing there.

- Very low count.
- Observations & Comments on the bottom.
- "No presence of current or former mold growth."
- But keep in mind very hard to count spores in house dust by Direct Microscopic Examination (DME.)
- Why? Spores are covered with dust!

Lift Tape of Surface Dust On The Top of My Cabinet. Nothing there.

IDENTIFICATION		Spores per cm^2	Percent of toal		Spores per cm^2	Percent of toal
Cladosporium						
Curvularla		3	60		4	67
Nigrospora						
Other Ascospores						
Other Basldospores		1	20		2	33
Pencillum Aspergillus						
Peronospora						
Pithomyces		1	20			
Torula						

We Also Tested For the Number of Viable (Live) Mold Spores From The AC

- Petri dishes filled with growth media were placed under an AC supply in my dining room.
- Collected viable/culture air samples for 10 minutes.

Only A Few Viable Spores From My Ducting Which is Extremely Clean

ANALYSIS METHOD	6120 Air Culturable			6120 Air Culturable			6120 Air Culturable		
LOCATION	OUTSIDE CONTROL			OUTSIDE CONTROL			CLEAN CONTROL MY HOUSE		
COC/LINE #	1258897-2			1258897-3			1258897-4		
SAMPLE TYPE & VOLUME	SETTLING			SETTLING			SETTLING		
SERIAL NUMBER	2			3			4		
COLLECTION DATE	Aug 5,2019			Aug 5,2019			Aug 5,2019		
ANALYSIS DATE	Aug 5,2019			Aug 5,2019			Aug 5,2019		
CONCLUSION	NOT ELEVATED			NOT ELEVATED			NOT ELEVATED		
IDENTIFICATION		Colonies	Percent of total		Colonies	Percent of total		Colonies	Percent of total
Aspergillus		5	36		5	71		2	40
Cladosporium		9	64		2	29		1	20
Peniillium									
TOTAL SPORES		14	100		7	100		5	100
MINIMUM DETECTION LIMIT		1			1			1	
BACKGROUND DEBRIS	Not Applicable			Not Applicable			Not Applicable		
OBSERVATIONS & COMMENTS									

Viable inside sample.

Viable Outside Controls (Of Course More Spore Growth)

Viable Outside Controls (Of Course More Spore Growth)

DNA Air Study Mold and Fragments (ARMI Panel Analyzed At Mycometrics)

LOCATION	Indoor clean control (my home)	Outside control
	Spore E./m3	**Spore E./m3**
Fungal ID / Sample ID	**#1 - A**	**#2 - GAR**
Alternaria alternata	ND	3
Aspergillus niger	ND	47
Aspergillus ochraceus	<1	3
Aspergillus penicillioides	1	1500
Aspergillus restrictus*	ND	45
Aspergillus sydowii	ND	230
Chaetomium globosum	ND	1
Cladosporium cladosporioides 1	1	43
Cladosporium herbarum	ND	ND
Eurotium (Asp.) amstelodami	ND	ND
Pacilomyces variotii	<1	<1
Penicillium chrysogenum	ND	2
Wallemia sebi	4	230

- Column #1: My house. Clean control. Nothing in the air sampling for approx. 24 hours at 22 lpm. Very large sample.
- Column #2: Outside. 2 hours at 22 lpm. Of course plenty mold.

Nasty coils. Clean or replace

DNA Dust Study Mold and Fragments (ARMI Panel Analyzed At Mycometrics)

LOCATION	Cabinet top dust (my home)
	Spore E./m3
Fungal ID / Sample ID	#5 - Cabinet Home
Alternaria alternata	7
Aspergillus niger	400
Aspergillus ochraceus	1
Aspergillus penicillioides	230
Aspergillus restrictus*	9
Aspergillus sydowii	92
Chaetomium globosum	15
Cladosporium cladosporioides 1	240
Cladosporium herbarum	5
Eurotium (Asp.) amstelodami	26
Pacilomyces variotii	57
Penicillium chrysogenum	46
Wallemia sebi	130

- Cabinet dust from my home. Plenty of mold in a tiny 2”x2” dust sample.
- Compare to surface dust sample analyzed by DME (discussed earlier) that found essentially nothing. Why?
- Because dust interferes with Direct Microscopic Examination but not with DNA analysis.

Swiffer Test for Cleanliness

- Swiffer testing of floor dust found nothing. Floor clean. If no dust, no spores in the dust.
- This is the result of carefully cleaning all floors 3x per week due to dog hair.

Conclusion #1. Case Study #1 Forget Surface Sampling

- Lift tape of surface dust on cabinet top examined with DME found nothing vs mold DNA testing which found mucho mold.
- Was this because the surface dust had few spores and mostly fragments or were most of the spores covered up by heavy dust and invisible to DME analysis but clearly detectable by DNA analysis for which dust does not interfere? Can't tell. But ...
- Conclusion #1: Forget lift tapes/swabs of surface dust analyzed by Direct Microscopic Exam (DME).

Conclusion #2: Surface Dust Does NOT = Exposure

- DNA testing of heavy dust on cabinet top which is rarely cleaned found mucho mold. But nothing in the air.
- DNA testing of isolated pockets of dust for mold always overestimates exposure. Forget testing pockets of surface dust by either DME or DNA.
- Conclusion #2: What is in pockets of dust in a clean home has nothing to do with mold in the air and nothing to do with exposure.

Nasty AC ducting. Clean or replace.

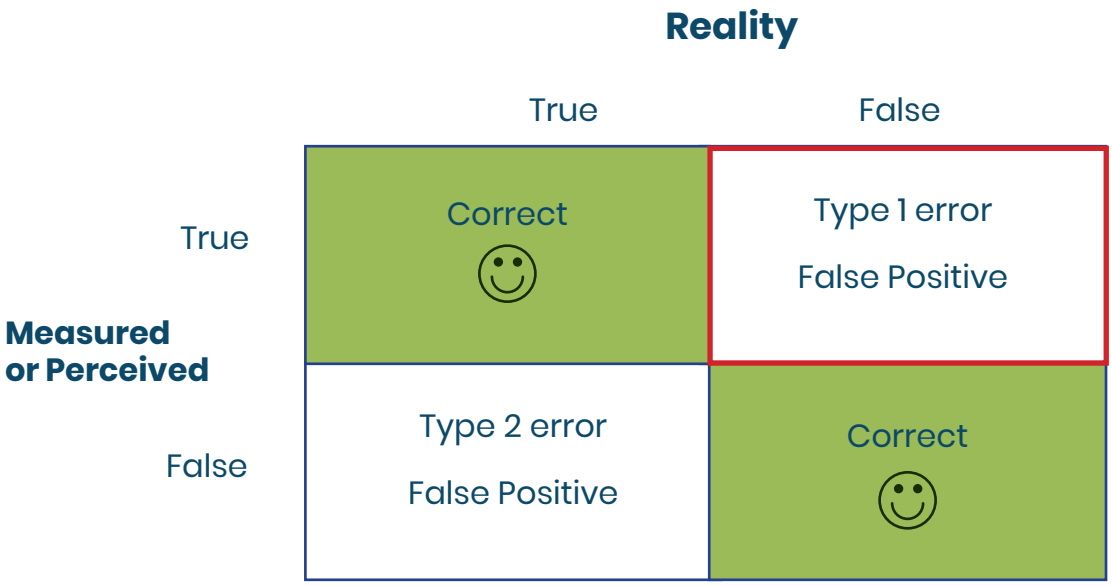
Conclusion #3 DNA Air Sampling = Total Exposure

- DNA air sampling measures both mold spores as well as mold fragments and found plenty of fragments while spore traps and culture testing that do not test for fragments, found nothing.
- Conclusion #3: Only air testing for mold/mold fragments using DNA methods provides a reliable measure of what is in the air … which means exposure.
- That does not mean that culture testing and spore trap testing should never be used.
- DNA air testing is expensive and requires overnight sampling. And only tests for a limited set of mold species.
- **Spore traps** detect any and all types of spores. With traps, if one finds elevated mold in the air then that's all you need to know to determine there is a mold exposure problem. With traps, if one does not find elevated mold in the air, this may be a false negative as usually it is mold fragments that are the major cause of irritation.
- **Culture testing** is also cheap. But takes 3-5 days to get results back. It detects any and all or most species of live spores. (Some do not grow on typical culture media. But most do.) Dead spores are invisible to culture testing. The ratio of culture test results when compared to spore traps will give a ratio of live versus total (dead+live). And because spores die over time, a high ratio of live versus total therefore means a more recent water event than does a low ratio. Testing outside gives a low ratio of viable to total. Most spores in the outside air are dead.

Clean Control Reference

- A super clean home with super clean floors and ducting and no wall to wall carpeting.
- With MERV 13 or better air filters. FAN = ON.
- Will have essentially no measurable mold spores or fragments in the air or floor dust.
- And there will be no irritation even for the most sensitive.
- **Use this case study on a clean control and compare to the numbers in your home.**

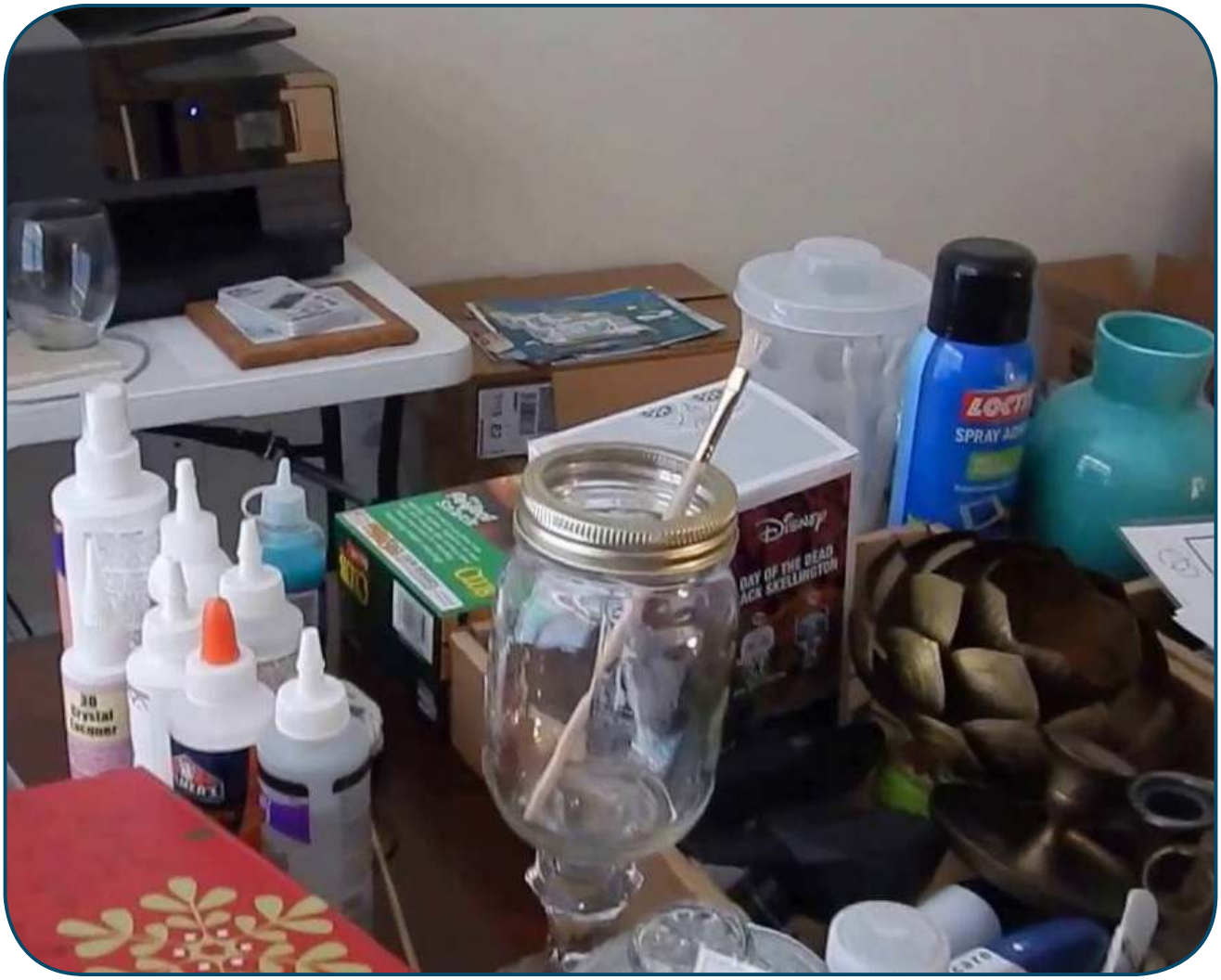
CASE STUDY: RB TYPE 1 ERROR.



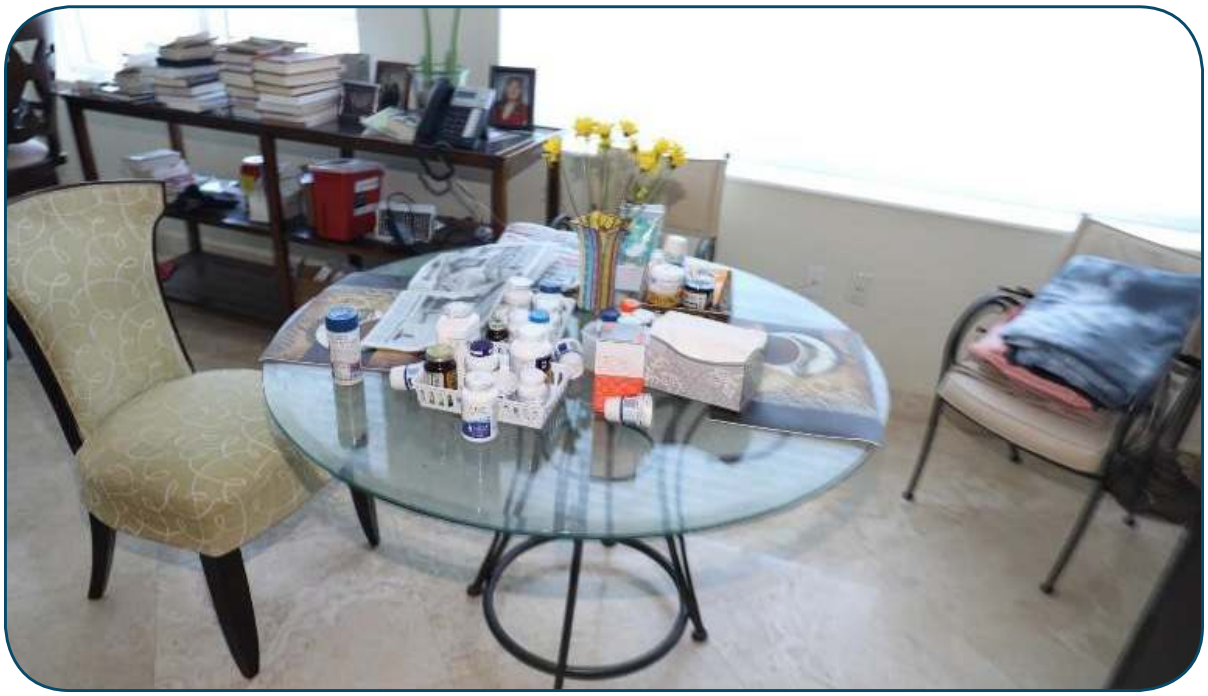
CASE STUDY: TYPE 1 ERROR. (FALSE POSITIVE) ROBERT B.

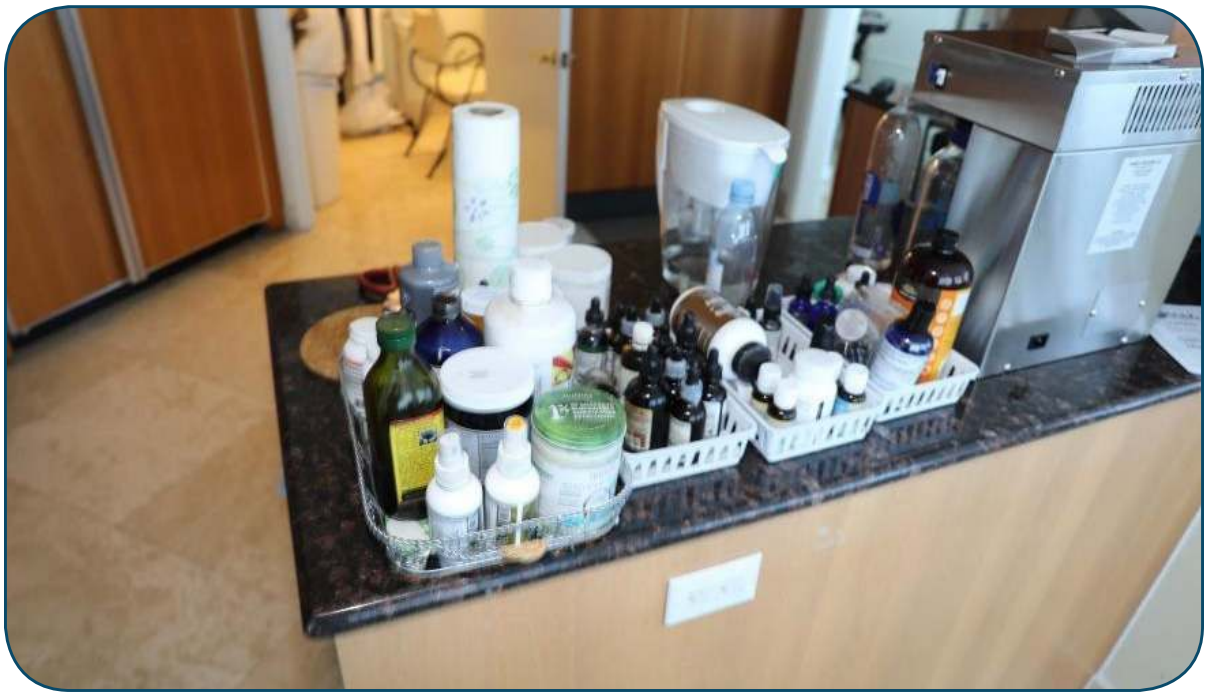
- Robert is being treated for CIRS and almost everything else imaginable.
- He's a very careful person and keeps his home clean and dry.
- Here's pictures of the medicines and vitamins he takes daily.

There is Some Clutter Lots of Medicines / Some Fabric



There is Some Clutter Lots of Medicines / Some Fabric





Case Study: Type 1 Error (False Positive) Robert B

- **Fallacy A:** Since Robert is not getting better with treatment for mold toxin exposure, his physicians believe the treatment is correct and if he is not getting better, he must be living in a moldy home.
- **Fallacy B:** As well, his urine was tested for mold toxins and results come back high indicating high levels of current mold toxin exposure. This gives further reason (to his physicians) that his home must be mold contaminated.
- To prove that there are no problems with the home and doctors are treating someone for mold illness that is not ill from current mold exposure and that urine testing for mycotoxins is bogus … is not easy to do. Doctors will fight you.
- Doctors have urine tests taken for mycotoxins because they always or almost always find high levels of toxins in urine. Such results are interpreted to mean continued exposure to mold toxins and so prove to the doctors that the reason the patient is not getting better is not because the therapy is not working but that there is continued exposure. Doctors do not want to believe that their treatment procedures make no sense or do not work. Hard (impossible?) to convince them otherwise.
- Ruling out mold exposure in a home in order to convince doctors that there is no current mold exposure and that their treatment makes no sense and that the urine tests are bogus …. takes a great deal more testing than ruling something in (finding something).
- **So we went a bit overboard in order to so prove. But in the long run, to no avail. His doctor would not change their opinion that Robert continued to stay sick because his home must be mold contaminated. He had to find another doctor. Turned out that was the best thing that could have happened. The patient started to improve.**

PROFESSIONAL MOLD ASSESSMENT BY LICENSED ASSESSOR/ HYGIENIST

Home is Well Cleaned

- The maid cleans RB's relatively new condo every week with a quality German-made HEPA Vac.
- No carpet.
- There are high quality, allergen-rated, Merv 13 filters in the 2 main ACs.
- The Phoenix Guardian air scrubber in the master is a True HEPA.
- Charcoal filter on top of air scrubber to collect any possible organic gases.
- No evidence of current mold or water problems.

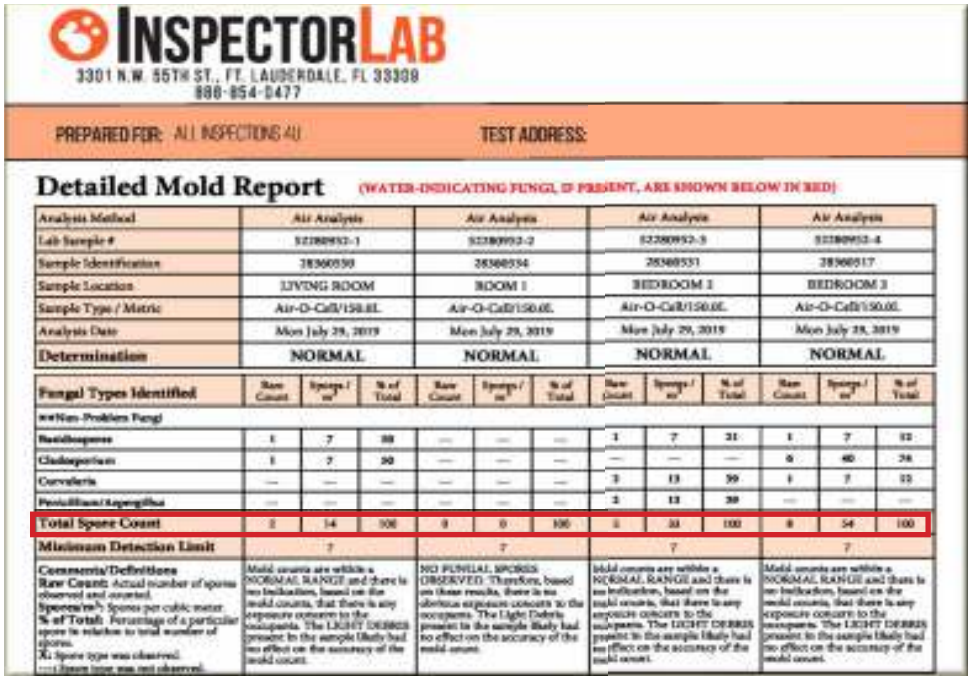
Doctors Insisting Home Full of Mold. Fix or Move Out.

- But his doctors were telling him that since urine testing and blood testing results show super high for mold toxins … there must be current mold exposure.
- There must be huge amounts of current mold toxin exposure, how else can you explain the labs?
- His doctors said: If you want to get better, fix the mold or move out.
- Doctors Kept Insisting: There must be huge amounts of current mold toxin exposure, how else can you explain the super high toxin levels measured in blood and urine?
- That's easy … the results from non-accredited labs with their proprietary procedures are bogus.
- But how do we prove that? That's easy …
- **If we prove that the home is completely free of mold exposure, then there is no current mold toxin exposure and therefore the lab results that are a measure of current exposure are bogus.**
- **Let the reader decide.**

Testing By Professional Mold Assessor/ Inspector (Ray)

- Robert hired a professional mold assessor (Ray) … a State of Florida licensed home inspector and State of Florida licensed mold assessor.
- Ray performed mold spore trap air sampling and performed an extensive visual inspection with a FLIR and moisture meter.
  - No mold found.
  - No water or water stains found.
  - No elevated moisture levels.

Air Sampling Results Extremely Low



INSPECTORLAB
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888-854-0477

PREPARED FOR: ALL INSPECTIONS 4U TEST ADDRESS:

Detailed Mold Report (WATER-INDICATING FUNGI, IF PRESENT, ARE SHOWN BELOW IN RED)

Analysis Method	Air Analysis	Air Analysis	Air Analysis	Air Analysis
Lab Sample #	S2280952-1	S2280952-2	S2280952-3	S2280952-4
Sample Identification	28360530	28360534	28360531	28360517
Sample Location	LIVING ROOM	ROOM 1	BEDROOM 2	BEDROOM 3
Sample Type / Matric	Air-O-Cell/150.0L	Air-O-Cell/150.0L	Air-O-Cell/150.0L	Air-O-Cell/150.0L
Analysis Date	Mon July 29, 2019	Mon July 29, 2019	Mon July 29, 2019	Mon July 29, 2019
Determination	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**

Fungal Types Identified	Raw Count	Spores/m³	% of Total	Raw Count	Spores/m³	% of Total	Raw Count	Spores/m³	% of Total	Raw Count	Spores/m³	% of Total
Non-Problem Fungi												
Basidiospores	1	7	88	—	—	—	1	7	21	1	7	12
Cladosporium	1	7	50	—	—	—	—	—	—	6	40	75
Curvularia	—	—	—	—	—	—	3	13	39	1	7	12
Penicillium/Aspergillus	—	—	—	—	—	—	2	13	39	—	—	—
Total Spore Count	2	14	100	0	0	100	6	33	100	8	54	100
Minimum Detection Limit		7			7			7			7	

Comments/Definitions
Raw Count: Actual number of spores observed and counted.
Spores/m³: Spores per cubic meter.
% of Total: Percentage of a particular spore in relation to total number of spores.
X: Spore type was observed.
---: Spore type was not observed.

Mold counts are within a NORMAL RANGE and there is no indication, based on the mold counts, that there is any exposure concern to the occupants. The LIGHT DEBRIS present in the sample likely had no effect on the accuracy of the mold count.

NO FUNGAL SPORES OBSERVED. Therefore, based on these results, there is no obvious exposure concern to the occupants. The Light Debris present in the sample likely had no effect on the accuracy of the mold count.

Mold counts are within a NORMAL RANGE and there is no indication, based on the mold counts, that there is any exposure concern to the occupants. The LIGHT DEBRIS present in the sample likely had no effect on the accuracy of the mold count.

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- Air sampling for mold spores … results show one of the cleanest homes ever tested.
- Visual inspection. Clean. Dry. Mold free.
- Why is the air so clean?
- See next pictures.

Merv 13 Air Filters in Both AC's & FAN Running 24x7

Phoenix Guardian True HEPA With Charcoal Filter in Master

Dehumidifier in Master

Clean/Mold Free?

- Appeared to be Clean. Dry. Mold Free. At least by visual inspection and spore trap testing.
- And we know that the HVAC System is also clean and mold free as it had been carefully disassembled and refurbished recently.
- But Robert's current urine and blood tests for mycotoxins were off the chart and the labs said that this must be from current exposure.
- Doctors insist lab testing valid. Must be huge amounts of mold in the home. Fix or move out.

Clean/Mold Free? Or Phony Lab Results?

- Were the lab results for elevated mycotoxins phony … as Dr. Shoemaker's recent study proves? (See Appendix A.)
- We think so, but how to prove experimentally?
    - The lab procedures were all proprietary from non-accredited labs one of which was in Mexico.
    - Never been Peer Reviewed/ published in professional Journals.
    - No double-blind controls.
- Clearly Robert was suffering, and traditional accepted medical tests for CIRS showed he was getting worse and not better.
- So there was a sense of urgency.

Clean/Mold Free? Or Not?

- Robert's health was getting worse.
- Was the problem that his doctors were treating for mycotoxin exposure where there was none (years of toxin binders and dozens of other treatments) …
- And the treatment was making him sick or was not appropriate.
- Or was there somehow a huge mold problem that eluded an experienced mold assessor? How do you know?

Nasty AC supply plenum.

Retesting Home. Including DNA Testing For Mold & Mold Fragments

- Of course we had to rule out by every means necessary current exposure to mold and mold toxins.
- So we retested the home using spore traps to double check Ray's work, as well as tested with a few other methods, including DNA testing for mold and mold fragments.
- **Ruling out mold exposure (proving no Type 1 Error) requires a lot of testing.**

Moisture Meter

OUR SPORE TRAP SAMPLING AT ROBERT'S

Laser Particle Counter Results

	1.0 micron	2.5 micron	5 micron	10 micron
Robert	9.4K	2.5K	.7K	.4K
Clean control My Home	2.4K	.1K	.06K	.05K

- Even though the particle count is elevated versus the clean non-cluttered control home (mine) the total particle level in Robert's home is still low (but not perfect.)
- We ran his spore trap samples for 10' versus 3-5' to improve collection accuracy.

There is Some Clutter Lots of Medicines / Some Fabric

- There is no visible dust on any surfaces anywhere in Robert's home.
- But there was certainly some fabric table cloths and many bottles of meds and vitamins... some clutter.
- This correlated with the not perfect laser particle count.

Mold Spore Trap (Air Sampling) Results. Very Low.

One of The Cleanest Homes Ever Tested

- Lab results for indoor spore trap air sampling for mold spores were similar to those found by Home Inspector Ray a week earlier.
- Shows one of the cleanest homes ever tested based on spore traps.
- Total spore counts at about 100 spore per cubic meter of air. That's unbelievably clean air (of mold spores).
- But the spore trap testing did indicate some dust in the air.

Mold Spore Trap Sampling Indoor Air Very Low. Not Elevated

ANALYSIS METHOD	6110 Air Direct Examination			6110 Air Direct Examination			6110 Air Direct Examination			6110 Air Direct Examination		
LOCATION	INDOOR			INDOOR			OUTDOOR CONTROL			OUTDOOR CONTROL		
COC/LINE #	1258904-1			1258904-2			1258904-3			1258904-4		
SAMPLE TYPE & VOLUME	AIR-O-CELL 100 - 150L			AIR-O-CELL 100 - 150L			AIR-O-CELL 100 - 150L			AIR-O-CELL 100 - 45L		
SERIAL NUMBER	28797249			28796291			28797154			28797168		
COLLECTION DATE	Aug 3,2019			Aug 3,2019			Aug 3,2019			Aug 3,2019		
ANALYSIS DATE	Aug 5,2019			Aug 5,2019			Aug 5,2019			Aug 5,2019		
CONCLUSION	NOT ELEVATED			NOT ELEVATED			CONTROL			CONTROL		
IDENTIFICATION	Raw Count	Spores per m^3	Percent of Total	Raw Count	Spores per m^3	Percent of Total	Raw Count	Spores per m^3	Percent of Total	Raw Count	Spores per m^3	Percent of Total
Arthrinium							1	7	1	1	22	1
Bipolaris/Drechslera	1	7	6									
Curvularia	1	7	6				3	20	2	1	22	1
Ganoderma							2	13	1			
Nigrospora										2	44	2
Other Ascospores	1	7	6	1	7	7	11	73	8	1	22	1
Other Basidiospores	3	20	19	3	20	20	6	40	4	2	44	2
Penicillium/Aspergillus	10	67	62	11	73	73	114	760	81	94	2,100	91
Smuts, myxomycetes							2	13	1			
Spegazzinia							1	7	1			
Torula										2	44	2
TOTAL SPORES	16	108	100	15	100	100	140	933	100	103	2,298	100
MINIMUM DETECTION LIMIT	1	7					1	7		1	22	
BACKGROUND DEBRIS	Moderate			Light			Moderate			Moderate		
Cellulose Fiber	2	13		1	7		2	13				

Indoor Air is Very Clean

Mold Spore Very Low. But Some Dust in The Indoor Air.

- **Lab results show some dust in the air as indicated by Background Debris = Moderate**

ANALYSIS METHOD	6110 Air Direct Examination			6110 Air Direct Examination			6110 Air Direct Examination			6110 Air Direct Examination		
LOCATION	INDOOR			INDOOR			OUTSIDE CONTROL			OUTSIDE CONTROL		
COC/LINE #	1258904-1			1258904-2			1258904-3			1258904-4		
SAMPLE TYPE & VOLUME	AIR-O-CELL 100 - 150L			AIR-O-CELL 100 - 150L			AIR-O-CELL 100 - 150L			AIR-O-CELL 100 - 45L		
SERIAL NUMBER	28797249			28796291			28797154			28797168		
COLLECTION DATE	Aug 3,2019			Aug 3,2019			Aug 3,2019			Aug 3,2019		
ANALYSIS DATE	Aug 5,2019			Aug 5,2019			Aug 5,2019			Aug 5,2019		
CONCLUSION	NOT ELEVATED			NOT ELEVATED			NOT ELEVATED			NOT ELEVATED		
IDENTIFICATION	Raw Count	Spores per m^3	Percent of Total	Raw Count	Spores per m^3	Percent of Total	Raw Count	Spores per m^3	Percent of Total	Raw Count	Spores per m^3	Percent of Total
Arthrinium							1	7	1	1	22	1
Bipolaris/Drechslera	1	7	6									
Curvularia	1	7	6				3	20	2	1	22	1
Ganoderma							2	13	1			
Nigrospora										2	44	2
Other Ascospores	1	7	6	1	7	7	11	73	8	1	22	1
Other Basidiospores	3	20	19	3	20	20	6	40	4	2	44	2
Penicillium/Aspergillus	10	67	62	11	73	73	114	760	81	94	2,100	91
Smuts, myxomycetes							2	13	1			
Spegazzinia							1	7	1			
Torula										2	44	2
TOTAL SPORES	16	108	100	15	100	100	140	933	100	103	2,298	100
MINIMUM DETECTION LIMIT	1	7		1	7		1	7		1	22	
BACKGROUND DEBRIS	Moderate			Light			Moderate			Moderate		
Cellulose Fiber	2	13		1	7		2	13				

One of The Cleanest Homes Ever Tested Based on Spore Traps

- But it is true that mold spore trap testing is not conclusive because it does not do a great job collecting small mold spores; is impacted by airborne dust for which there was some; **and spore trap testing does not detect mold fragments.**
- We followed this testing with other types of testing to double/ triple check the home for cleanliness focused on indoor air cleanliness.

VIABLE SPORE TRAP SAMPLING

Viable (Air Sampling) Results. Very Low.

Viable (Air Sampling) Results. Very Low.

- And we tested for viable spores coming out of Robert's AC ducting.
- Collected air sample for 10 minutes. Very low

Tested For the Number of Viable/ Culture (Live) Mold Spores in the Air

ANALYSIS METHOD	6120 Air Culturable			6120 Air Culturable			6120 Air Culturable			6120 Air Culturable		
LOCATION	RB AIR DUCTING			OUTSIDE CONTROL 8/2			OUTSIDE CONTROL 8/1			CLEAN CONTROL MY HOUSE		
COC/LINE #	1258897-1			1258897-2			1258897-3			1258897-4		
SAMPLE TYPE & VOLUME	SETTLING			SETTLING			SETTLING			SETTLING		
SERIAL NUMBER	1			2			3			4		
COLLECTION DATE	Aug 5,2019			Aug 5,2019			Aug 5,2019			Aug 5,2019		
ANALYSIS DATE	Aug 5,2019			Aug 5,2019			Aug 5,2019			Aug 5,2019		
CONCLUSION	NOT ELEVATED			NOT ELEVATED			NOT ELEVATED			NOT ELEVATED		
IDENTIFICATION		Colonies	Percent of Total		Colonies	Percent of Total		Colonies	Percent of Total		Colonies	Percent of Total
Aspergillus					5	36		5	71		2	40
Cladosporium					9	64		2	29		1	20
Peniillium												
TOTAL SPORES			100		14	100		7	100		5	100
MINIMUM DETECTION LIMIT		1			1			1			1	
BACKGROUND DEBRIS	Not Applicable			Not Applicable			Not Applicable			Not Applicable		
OBSERVATIONS & COMMENTS	No Fungi Detected											

- #1. RB ducting compared to ; Outside controls; and Clean Control (My House.)
- All very very low.

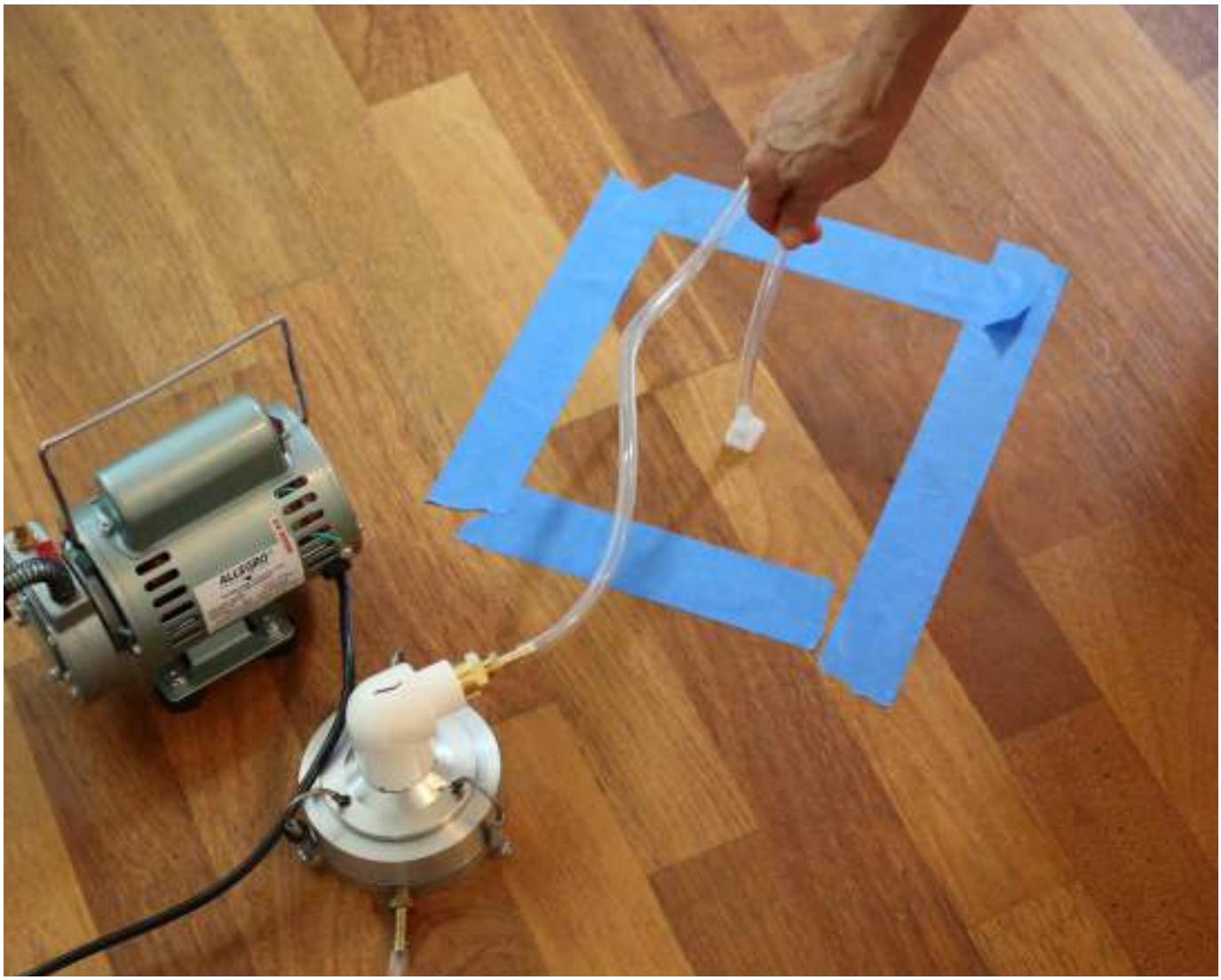
No viable mold spores found coming out of Robert's AC ducting.

Outside controls. Mold of course and plenty of stuff growing that is not mold so not listed on the mold report.

Viable Testing Summary

- Viable/ Culture testing is less susceptible to dust than spore trap testing.
- But as with spore trap testing, does not measure mold spore fragments that many consider more of a concern for CIRS clients than spores.
- Robert's home is on the water in Ft Lauderdale. Typically very little mold in the outside air because air is coming from the ocean breeze which has little to now mold.
- Robert's house very clean of viable mold spores. Confirms results from spore traps (viable + non-viable spores.)
- Not a popular procedure. But we like it. Use it.
- Viable (culture) testing is a very good way to visually show the level of spores in the air by taking photos of the petri dishes after 48-72 hours of incubation before taking to the lab.
- Compare outside air to air both inside the test home as well as in a clean control such as our home.
- The fact that the outside control shows mold is useful in validating that there is nothing wrong with the procedure or with the culture media.

SURFACE SAMPLING ANALYZED BY DIRECT MICROSCOPIC EXAMINATION (DME)



Lift Tape of Surface Dust on Top of RB's Cabinet in Laundry Room (No pix)

IDENTIFICATION		Spores per cm^2	Percentage of Total		Spores per cm^2	Percentage of Total
Arthrinium						
Bipolaris/Drechslera						
Curvularia						
Ganoderma						
Nigrospora						
Other Ascospores		1	20		2	33
Other Basidiospores						
Penicillium/Aspergillus		1	20		2	33
Smuts, myxomycetes					2	33
Spegazzinia						
Torula						
TOTAL SPORES						
MINIMUM DETECTION LIMIT						
BACKGROUND DEBRIS	Not Applicable			Not Applicable		
Observation and Comments	No presence of current or former growth observed. Only normally settled spores observed.			No presence of current or former growth observed. Only normally settled spores observed.		

Lift Tape of Surface Dust on Top of RB's Cabinet in Laundry Room (No pix)

Observation and Comments	No presence of current or former growth observed. Only normally settled spores observed.	No presence of current or former growth observed. Only normally settled spores observed.

- Very low count. "No presence of current or former mold growth".
- But keep in mind, such a test may drastically underestimate the mold spores present, as spores on surfaces will be embedded in / covered up by dust.

- This will become clear when we do the same surface dust test using DNA procedures (coming up) that will find thousands of mold spores or fragments in the same amount of dust.

Section Summary: Lift Tape (Or Swab) of Surface Dust.

- Lift tapes or swabs. Not a useful procedure for determining spore counts.
- Lift tapes or swabs are generally taken for pre- remediation testing of visible mold for the purpose of generating extra income by performing unnecessary testing or …
- Because insurance carriers often want testing of obvious / visible mold.

SURFACE TESTING FOR CLEANLINESS BY WHITE GLOVE TEST

White Glove Dust Test With Swiffer Robert's Floors

- Use a Swiffer to "re-clean" areas in Robert's home.
- Check for dirt on the Swiffer.

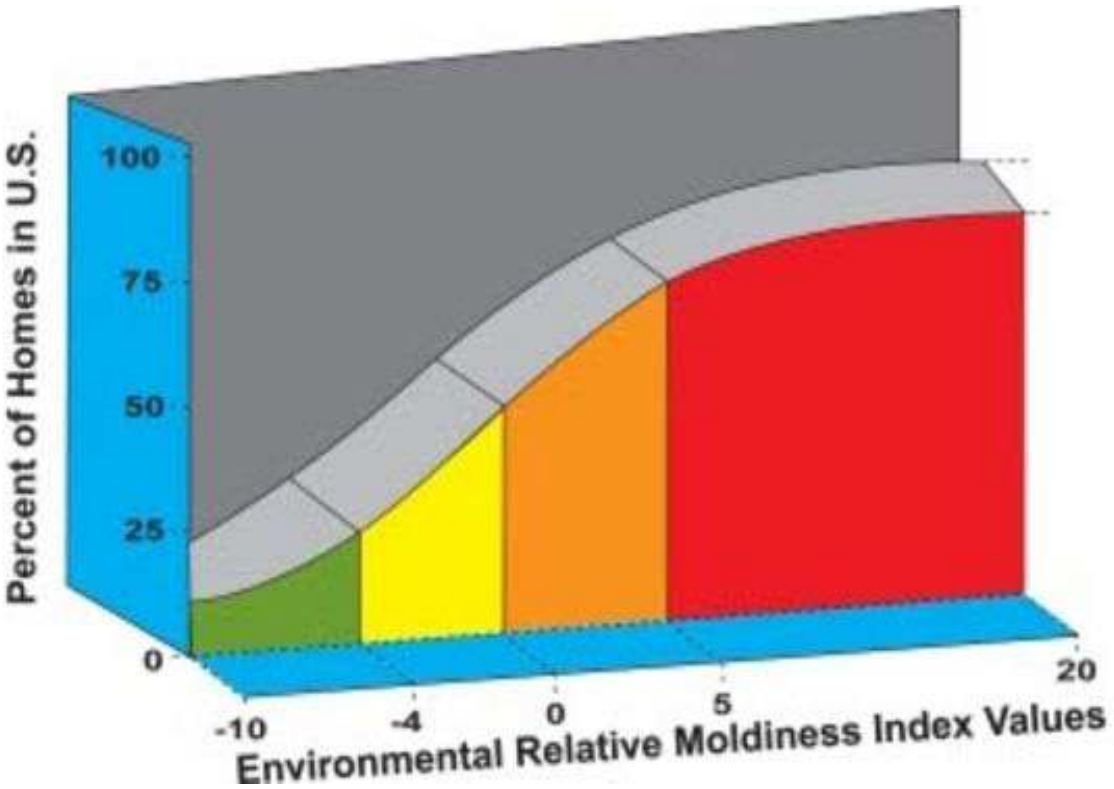
Swiffer Test Robert's Floors

- No trace of dust in Living Room or Dining Room or Kitchen floors or furniture surfaces. We extensively Swiffered (if that's a word!)
- But picture shows there is dust under Robert's office desk where it is not possible for the housekeeper to vacuum. She needs to be provided with Swiffer that can go places a vacuum cannot.

Swiffer (White Glove) Clearance Test

- In our experience, the Swiffer test is by far the best method for checking the settled dust for spores on flooring and content.
- For checking how clean is clean. No dust = No spores.
- If there is dust/discolored Swiffer … continue cleaning.
- Very easy to do. No waiting for lab results. No possibility for error.
- For some unknown reason not a popular test. My guess is that it is not popular because you cannot easily charge for it compared to taking samples.

ARMI DNA STUDY AT ROBERT'S HOME



ARMI DNA Study of Robert's Condo Clean & Outside Controls

LOCATION	Indoor clean control (my home)	Outside control
	Spore E./m3	Spore E./m3
Fungal ID / Sample ID	#1 - A	#2 - GAR
Alternaria alternata	ND	3
Aspergillus niger	ND	47
Aspergillus ochraceus	<1	3
Aspergillus penicillioides	1	1500
Aspergillus restrictus*	ND	45
Aspergillus sydowii	ND	230
Chaetomium globosum	ND	1
Cladosporium cladosporioides 1	1	43
Cladosporium herbarum	ND	ND
Eurotium (Asp.) amstelodami	ND	ND
Pacilomyces variotii	<1	<1
Penicillium chrysogenum	ND	2
Wallemia sebi	4	230

- Column #1: My house. Clean control. Nothing in the air sampling for approx. 24 hours at 22 lpm. Very large sample.
- Column #2: Outside. 2 hours at 22 lpm. Of course plenty mold.

ARMI/DNA Study of Robert's Condo

LOCATION	Cabinet top dust RB
	Spore E./mg
Fungal ID / Sample ID	**#7 - Cabinet Home**
Alternaria alternata	<1
Aspergillus niger	100
Aspergillus ochraceus	<1
Aspergillus penicillioides	180
Aspergillus restrictus*	ND
Aspergillus sydowii	200
Chaetomium globosum	19
Cladosporium cladosporioides 1	15
Cladosporium herbarum	2
Eurotium (Asp.) amstelodami	13
Pacilomyces variotii	12
Penicillium chrysogenum	240
Wallemia sebi	570

2x2 piece of Swiffer used to collect dust on top of Robert cabinet.

- Dust from top of cabinet in Robert's laundry room. Plenty of mold/mold spores in a tiny 2"x2" dust sample.
- Compare to surface dust analyzed by DME that found almost nothing. Why? Because dust covers up mold spores.

ARMI/DNA Study of Robert's Condo Duplicate Air Samples.

Fungal ID / Sample ID	#3 - R1	#4 - R2
Alternaria alternata	ND	ND
Aspergillus niger	<1	<1
Aspergillus ochraceus	ND	ND
Aspergillus penicillioides	ND	1
Aspergillus restrictus*	ND	ND
Aspergillus sydowii	ND	ND
Chaetomium globosum	ND	<1
Cladosporium cladosporioides 1	1	1
Cladosporium herbarum	<1	ND
Eurotium (Asp.) amstelodami	ND	ND
Pacilomyces variotii	<1	ND
Penicillium chrysogenum	ND	ND
Wallemia sebi	<1	ND

- DNA Air sampling. Duplicates. Approx 24 hours at 22 lpm. Nothing there. No mold. No spores. No fragments in the air. NO MOLD EXPOSURE.
- **Heavy mold in isolated pockets of surface dust. But nothing in the air.**

Proof That Mold In Dust Does Not Represent Exposure

- Here we have taken DNA air samples overnight. (Versus 10 minute with spore traps.)
- If there were any aerosolization of mold in dust as a result of normal activities moving around the home, that would be captured in the overnight air sample.
- Taking samples of pockets of dust for DNA analysis **has nothing to do with mold in the air and therefore exposure.**
- Nothing to do with inhaling/ breathing mold.
- Such dust testing should never be done.
- It is a waste of time and money.
- **Such testing overestimates exposure and is used as a scare tactic by mold assessors (and dare I say it …. Many physicians that prescribe it).**

Proof That Blood & Urine Testing Are Bogus

- Visually super clean and dry home.
- Testing shows no mold or mold fragment exposure in Robert's home.
- Therefore no mycotoxin exposure.
- The Lab readings from blood as well as urine testing showing high levels of continuous exposure to toxic mold **are erroneous**... because there is no current mold or mold toxin exposure.

DOCTORS ARE NOT MOLD ASSESSORS

YOUR DOCTOR KNOWS ABSOLUTELY NOTHING ABOUT MOLD TESTING or LAB TESTING FOR MYCOTOXINS.

YOUR HEALTH DEPENDS ON ACCEPTING THIS FACT.

TRUST YOUR DOCTOR BUT NOT ABOUT MOLD OR MOLD TOXIN TESTING.

Bad Data = Bad Decisions

- Bad Data on mold exposure can lead to bad decisions.
- Thinking there is mold exposure when there is not (Type 1 Error) based on:
  1. Bad data from non-accredited laboratories and/or
  2. Proprietary HERTSMI-2 sampling techniques.
- Can result in bad medical decisions.
- Bad data from Type 1 Error can result in:
  3. Unnecessary remediation expense;
  4. Resultant incorrect treatment causing illness and/or even death.
- Next we focus on #4.

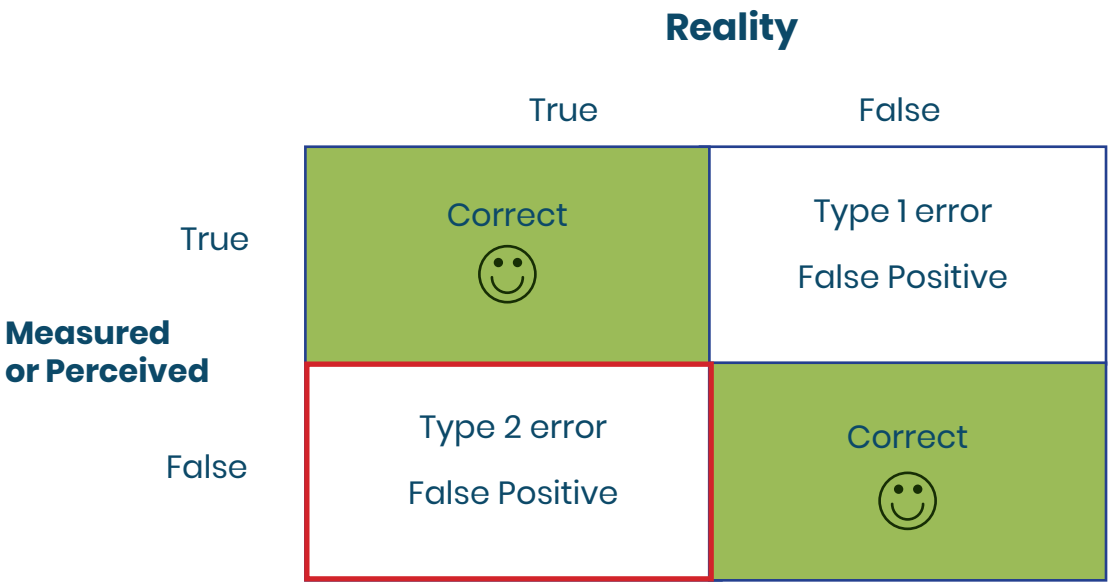
There Are No Mold Toxin Binders!

- Binders such as Cholestyramine or Welchol are not just "mold toxin" binders. That would imply they are specific for mold toxins. But they are not.
- Cholestyramine, Welchol and other binders, bind to hundreds or thousands of compounds in the human body.
- Many of these compounds are essential to health, but they are removed from the body by binders.
- What kind of compounds/chemicals are we talking about?

What Compounds Are Essential for Health But Removed By Binders?

- **Bile acids** are removed by binding agents.
- When bad lab data shows high levels of mold toxins in the blood or urine of people on toxin binders …
- Of course, the Doctor increases the frequency and amount of toxin binders.
- Over time, these high levels & long term usage of bile sequestrants can cause serious problems with the bowels. See:
- https://www.mayoclinic.org/drugs-supplements/cholestyramine-oral-route/side-effects/drg-20068562
- **Hormones and Vitamins** are removed by binding agents per the National Institute of Health. See: https://livertox.nih.gov/Cholestyramine.htm
- "Because cholestyramine can interfere with the absorption of other medications or vitamins, it may affect the levels of medications used for liver [or other] disease."
- "These effects are particularly important for **vitamins** A, D, E, K, and for hormones such as estrogens, corticosteroids, and thyroid hormone, and medications such as thiazide diuretics, acetaminophen and digoxin."
- **Long term incorrect or inappropriate toxin binding treatment can cause illness and/or even death.**

CASE KAREN S. TYPE 2 ERROR



Cry For Help

Inspection inbox

Karen S. <kcsxxxx@yahoo.com> to info Thu, Apr 4, 5:58 PM

Hi,

I am in the process of purchasing a home and had my inspection done today but am in need of a second opinion. The "mold expert" spent 15 minuets running through the house with a water meter and gave me a lecture that my symptoms are in my head and my ERMI is completely invalid. He did tell me that the house has an active leak in the master which the regular inspector found. That cost me $450. I did an ERMI through Envirobiomics with a score of 15.2 Q4 and a HERTSMI-2 score of 16. I am undergoing treatment for mold illness and am desperately seeking an experts opinion. It is very hard finding someone who truly understands all of this. I lost a house last week to mold because the seller did not disclose that there was a black mold problem with remediation and thankfully I found it with an ERMI (after spending $2000 on inspections). I wanted to see ifthere is any chance you are available anytime on Saturday for a mold inspection. I have until Monday to make a decision about this house. I live in Weston. I really like this house but need to know that it's a safe environment to live in.

Thank You,

Karen S.

Previous Inspections

- What do Karen's previous inspections mean?
- In one case a mold expert did a walk through and said there is no mold (and she's a nutcase.)
- In another case her doctor told her to get a DNA (ERMI) test of the house surface dust and it came back off the chart high.
- They can't both be right.

Karen Read My Analysis of DNA Dust Testing on Mold-Toxins.com

- Karen had called me after reading my article on DNA surface dust testing concerns at www.Mold-Toxins.com (my web site.)
- Very few people challenge the medical community's cult like faith in DNA testing floor dust.
- Fortunately, we were practically neighbors! So I stopped by and we chatted.
- Karen had just purchased a home for over $1M but she could not spend more than a few minutes in the home before getting ill.
- She has CIRS and is very sensitive to mold.
- She needed a though Home Inspection/Mold Assesment.
- A thorough Mold Assessment using the Team approach was not cheap but she just bought a house that she could not live in. Cheaper than the alternatives!

Mold Spore Trap Sampling Indoor Air Very Low. Not Elevated

- Spore traps results. Very low but not as low as the clean control. Approx 100 to 200.

ANALYSIS METHOD	6110 Air Direct Examination			6110 Air Direct Examination			6110 Air Direct Examination			6110 Air Direct Examination		
LOCATION	INDOOR PRE			INDOOR PRE			INDOOR PRE			INDOOR PRE		
COC/LINE #	1229714-1			1229714-2			1229714-3			1229714-4		
SAMPLE TYPE & VOLUME	AIR-O-CELL 100 - 45L			AIR-O-CELL 100 - 45L			AIR-O-CELL 100 - 45L			AIR-O-CELL 100 - 45L		
SERIAL NUMBER	27844012			27844023			27843996			27844004		
COLLECTION DATE	Apr 22,2019			Apr 22,2019			Apr 22,2019			Apr 22,2019		
ANALYSIS DATE	Apr 23,2019			Apr 23,2019			Apr 23,2019			Apr 23,2019		
CONCLUSION	NOT ELEVATED			NOT ELEVATED			NOT ELEVATED			NOT ELEVATED		
IDENTIFICATION	Raw Count	Spores per m^3	Percent of total	Raw Count	Spores per m^3	Percent of total	Raw Count	Spores per m^3	Percent of total	Raw Count	Spores per m^3	Percent of total
Chaetomium	1	22	12									
Cladasporium							6	130	86			
Other Ascopores	1	22	12							1	22	25
Other Basidiospores	3	67	38	2	44	33	1	22	44	3	67	75
Penicillium/Aspergillus	3	67	38	4	89	67						
TOTAL SPORES	8	178	100	6	133	100	7	152	100	4	89	100
MINIMUM DETECTION LIMIT	1	22		1	22		1	22		1	22	
BACKGROUND DEBERIS	Light			Light			Light			Light		
Cellulose Fiber	1	22		1	22		1	22		1	22	
OBSERVATION & COMMENTS												

- Indoor pre. Quadruplicate samples. Background debris. Light. No airborne dust.

BACKGROUND DEBERIS	Light			Light			Light			Light		
Cellulose Fiber	1	22		1	22		1	22		1	22	
OBSERVATION & COMMENTS												

- No content. No dust. No furniture. She had just closed on the empty home.
- Swiffer test for floor dust. Clean as a whistle. No dust.
- Quadruplicate spore trap tests. Same room. 3 minutes apart. All very low.
- And all with Light Background debris. Confirming clean.
- But Spore Trap results not as low as in the Clean Control home. Why not? Let's find out.

Visual assessment of the home interior … home appeared to be pristine

- Yet Karen was sick in the home. Who do you trust?
- In our experience the occupant that says they are sick in the home but not outside is never wrong.
- There is always a major problem of significant exposure somewhere. It is simply hidden from view. So look!

Mold hidden behind air handler.

- If the home is very clean. No old carpets. Not 90 years old. And with no active leaks. And not a rental unit where the landlords cover up/hide problems.
- The irritation/exposure is probably AC/Ducting related.

Pre-Remediation Culture Sampling (Quadruplicates)

- We repeated the air testing using culture/viable mold testing to compare to spore trap results.
- Again, culture tests for live/viable mold spores only (vs spore traps for both dead + alive.)
- Dead spores are invisible to culture testing.
- Similar numbers to Spore Traps results. Very low.

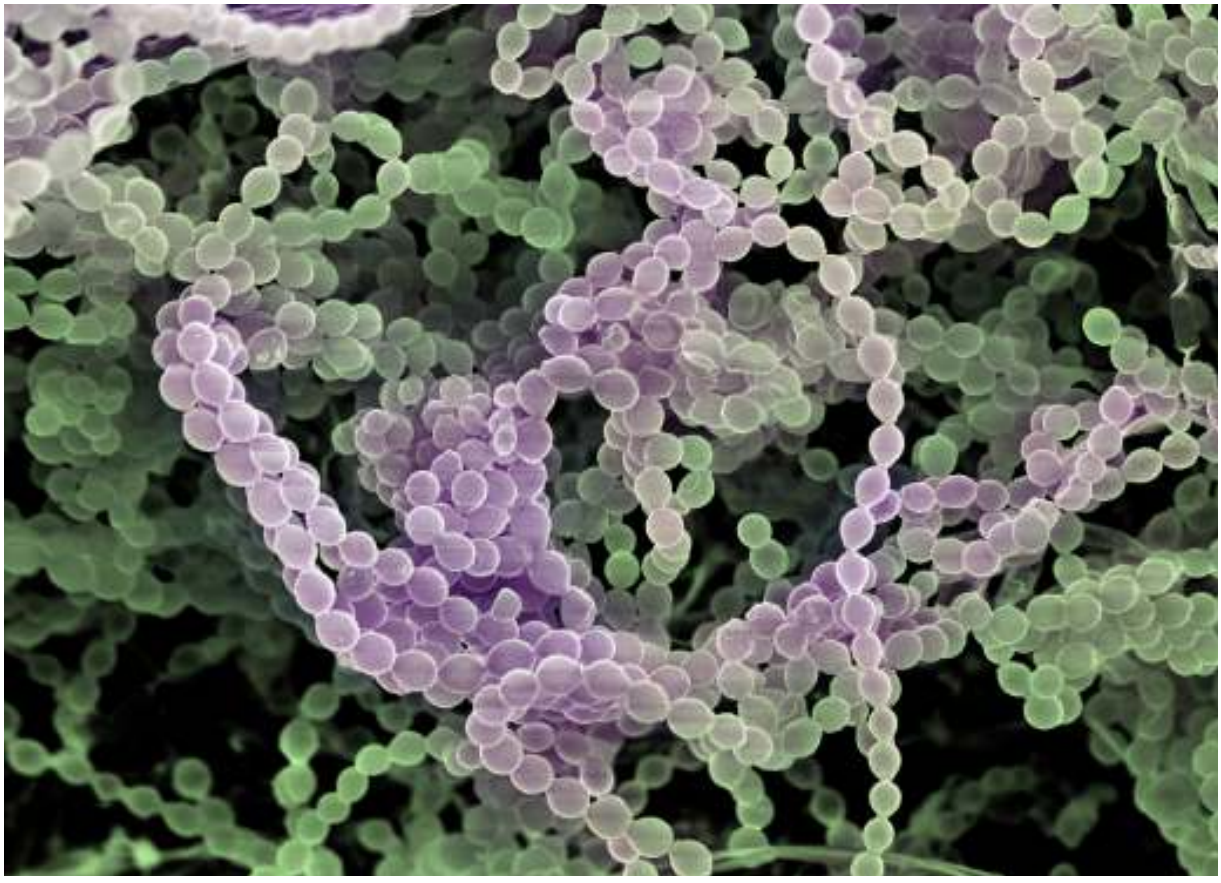
ANALYSIS METHOD	Culture Sample			Culture Sample			Culture Sample			Culture Sample		
LOCATION	INSIDE PRE 4/22			INSIDE PRE 4/22			INSIDE PRE 4/22			INSIDE PRE 4/22		
COC/LINE #	1231056-1			1231056-2			1231056-3			1231056-4		
SAMPLE TYPE & VOLUME	AIR IMPACTION- 56L			AIR IMPACTION- 56L			AIR IMPACTION- 56L			AIR IMPACTION- 56L		
SERIAL NUMBER	1			2			3			4		
COLLECTION DATE	Apr 22,2019			Apr 22,2019			Apr 22,2019			Apr 22,2019		
ANALYSIS DATE	Apr 29,2019			Apr 29,2019			Apr 29,2019			Apr 29,2019		
CONCLUSION	NOT ELEVATED			NOT ELEVATED			NOT ELEVATED			NOT ELEVATED		
IDENTIFICATION	Raw Count	CFU per m^3	Percent of total	Raw Count	CFU per m^3	Percent of total	Raw Count	CFU per m^3	Percent of total	Raw Count	CFU per m^3	Percent of total
Aspergillus				1	18	10				1	18	17
Chrysosporium										1	18	17
Cladosporium	3	54	30	7	120	69	2	36	13	4	71	66
Curvularia												
Non-sporulating fungi	6	110	60	2	36	21	3	54	20			
Penicillium	1	18	10				10	180	67			
TOTAL SPORES	10	182	100	10	174	100	15	270	100	6	107	100
MINIMUM DETECTION LIMIT	1	18		1	18		1	18		1	18	
BACKGROUND DEBERIS	Not Applicable			Not Applicable			Not Applicable			Not Applicable		
OBSERVATION & COMMENTS												

Culture results. Low. But very similar in number to spore traps. Means that the few spores found are newer/ live highly irritating spores.

- Culture results low. But very similar in number to spore traps that measure live + dead.
- Indicates that the mold spores found by spore traps are mostly fresh/ new spores.
- Since live/ fresh spores are more irritating than dead spores as they can (try to) colonize sinus cavities, when you find that spores in the air are mostly new/live …
- That is cause of concern.

Pre-Remediation DNA Sampling Dust/Air

- We took 4 pre-remediation DNA mold tests. Sent to Mycometrics for ARMI analysis.
  1. Surface dust on top of Karen's kitchen cabinets.
  2. Indoor air in the home.
  3. Indoor air in known clean control home (our home.)
  4. Surface mold from the moldy AC fiberglass supply plenum.



Pre-Remediation DNA Sampling Kitchen Dust

- Collected small amount of dust on the top of Karen's kitchen cabinets which required a ladder to reach.
- Plenty of mold present in this isolated (never cleaned) dust.
- (No floor dust at all.)
- Place was empty and visually very clean.

LOCATION	Kitchen Cabinet Dust
	Spore E./mg
Fungal ID / Sample ID	**#1**
Aspergillus Niger	19
Aspergillus Penicillioides	270
Aspergillus Restrictus*	1
Aspergillus Sclerotiorum	1
Aspergillus Sydowi	ND
Aspergillus Ungius	4
Aspergillus Vesicolor	71
Chaetomium globosum	15
Cladosporium sphaerospermum	7
Eurotium (Asp.) amstelodami*	4
Paecilomyces variotii	4
Penicillium variabile	26
Scopulariopsis chartarum	1
Stachybotrys chartarum	<1

Pre-Remediation DNA Sampling Dust On AC Plenum

- Mold on surface (growing on) moldy AC plenum in the garage.
- Lots of Cladosporium mold.

LOCATION	AC Supply Plenum Dust
	Spore E./mg
Fungal ID / Sample ID	**#2**
Aspergillus Niger	1
Aspergillus Penicillioides	35
Aspergillus Restrictus*	ND
Aspergillus Sclerotiorum	19
Aspergillus Sydowi	8
Aspergillus Ungius	ND
Aspergillus Vesicolor	30
Chaetomium globosum	2
Cladosporium sphaerospermum	160000
Eurotium (Asp.) amstelodami*	ND
Paecilomyces variotii	2
Penicillium variabile	2
Scopulariopsis chartarum	ND
Stachybotrys chartarum	1

Pre-Remediation Group 1 ARMI/DNA Sampling Air

- 1st column. DNA air sample. Karen home. Overnight. Quite high compared to clean control 2nd column.
- Some Stachybotrys (toxic).
- Compare to almost nothing found in the spore trap or culture air testing.
- Is this due to mold fragments that are only found with DNA testing but not with spore traps or culture testing?

LOCATION	Pre Remediation 25 hrs at 22 LPM (Air)	Clean Control 18 hrs at 22 LPM (Air)
	Spore E./mg	Spore E./mg
Fungal ID / Sample ID	**#4**	**#3**
Aspergillus Niger	1	ND
Aspergillus Penicillioides	84	ND
Aspergillus Restrictus*	6	ND
Aspergillus Sclerotiorum	1	ND
Aspergillus Sydowi	27	ND
Aspergillus Ungius	1	ND
Aspergillus Vesicolor	18	ND
Chaetomium globosum	4	ND
Cladosporium sphaerospermum	45	<1
Eurotium (Asp.) amstelodami*	1	<1
Paecilomyces variotii	1	ND
Penicillium variabile	13	ND
Scopulariopsis chartarum	3	ND
Stachybotrys chartarum	3	ND

- Lab results for air sampling for mold spores by both Spore Trap and Culture in Karen's home show a super clean home.
- DNA testing of house air finds high results.
- Is there a problem with the testing?
- **Why the apparently conflicting data?**
- **Answer: This pattern is always caused by mold fragments being released from the AC system, plenums, or ducting as a result of high air flow in contact with hidden (dead or live) mold within the HVAC System.**
- **The mold fragments are invisible to spore trap and viable/culture testing but not to DNA air testing as the fragments contain mold DNA.**

Why Pre-Remediation DNA Air Sampling Elevated?

- Well that sounds great in theory.
- But that cannot be right because the ACs in Karen's house are brand new!
- WRONG! AC contractor replaced the AC coils but left the rest of the HVAC system filthy. We then carefully cleaned. And then retested. See pictures that follow.

AC Contractors ALWAYS Solve Irritation/Odor By Selling a New AC

AC Contractors ALWAYS Solve Irritation/Odor By Selling a New AC


Black mold in supply plenum over the new Acs.


Filthy inside the return air boxes under the new ACs.

Filthy return air ducting.

Out with the dirty/old. In with the new.

Putting In MERV 13 Air Filter Cut to Size

HVAC System. Cleaned / Refurbished.

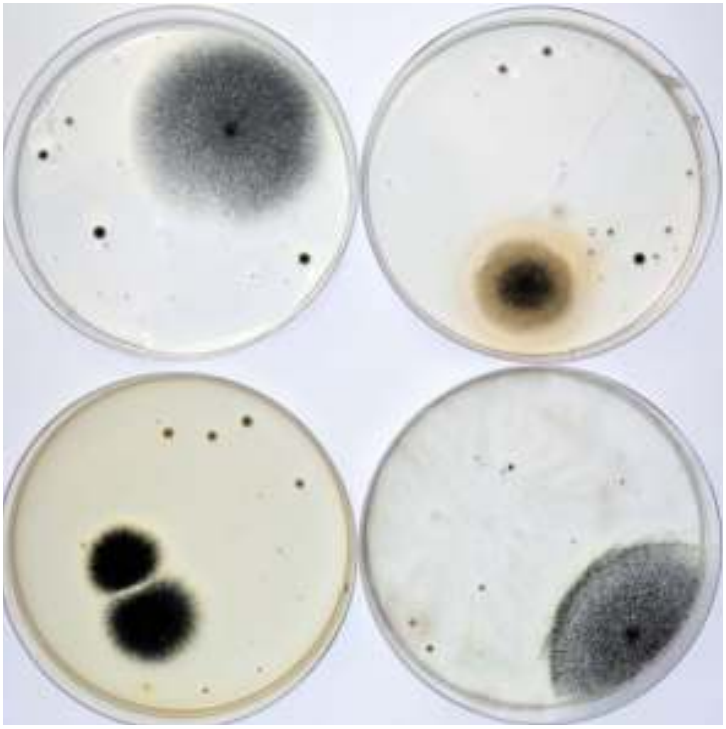
- After the intrusive inspection found massive problems in the AC and ducting we rebuilt the AC return air boxes and refurbished all the duct lining.
- We then recleaned all the floors and shelving followed by visual testing with new Swiffers to make sure there was no detectable remaining dirt on the floors/dust.
- We installed Merv 13 rated air filters in the returns and ran the ACs with the FAN=ON overnight.
- We then performed Post Remediation Verification testing. Results follow. All perfect.

Post Remediation Spore Trap Sampling

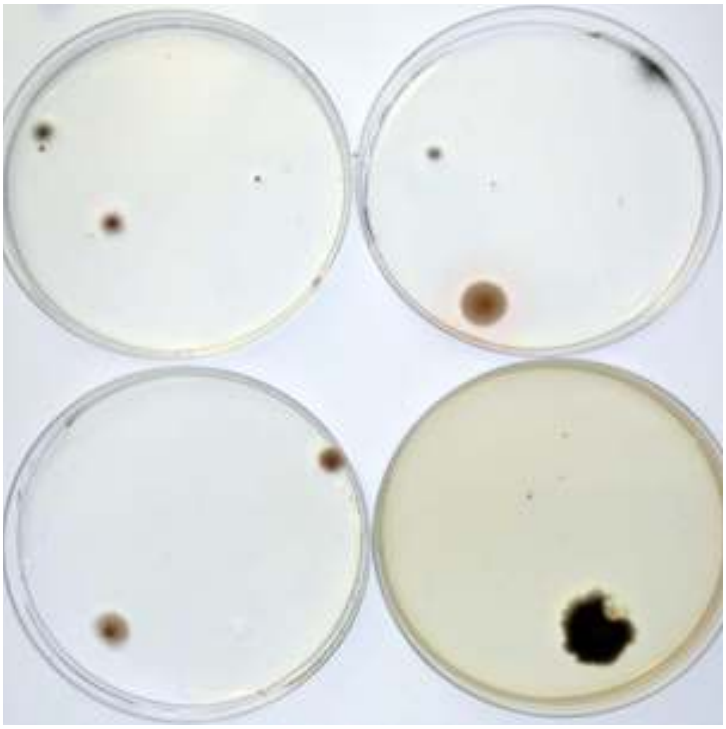
ANALYSIS METHOD	6110 Air Direct Examination			6110 Air Direct Examination			6110 Air Direct Examination			6110 Air Direct Examination		
LOCATION	INDOOR POST 1			INDOOR POST 2			INDOOR POST 3			INDOOR POST 4		
COC/LINE #	1230513-1			1230513-2			1230513-3			1230513-4		
SAMPLE TYPE & VOLUME	AIR-O-CELL 100 - 45L			AIR-O-CELL 100 - 45L			AIR-O-CELL 100 - 45L			AIR-O-CELL 100 - 45L		
SERIAL NUMBER	26088512			25900448			25906627			26078050		
COLLECTION DATE	Apr 25,2019			Apr 25,2019			Apr 25,2019			Apr 25,2019		
ANALYSIS DATE	Apr 25,2019			Apr 25,2019			Apr 25,2019			Apr 25,2019		
CONCLUSION	NOT ELEVATED			NOT ELEVATED			NOT ELEVATED			NOT ELEVATED		
IDENTIFICATION	Raw Count	Spores per m^3	Percent of total	Raw Count	Spores per m^3	Percent of total	Raw Count	Spores per m^3	Percent of total	Raw Count	Spores per m^3	Percent of total
Chaetomium												
Chlamydospores												
Cladasporium				1	22	100				3	67	60
Curvularia												
Ganoderma	2	44	50				1	22	20			
Other Ascospores	2	44	50				2	44	40	1	22	20
Other Basidiospores							2	44	40	1	22	20
Penicillium/Aspergillus												
Smuts, myxomycetes												
Torula												
TOTAL SPORES	4	88	100	1	22	100	5	110	100	5	111	100
MINIMUM DETECTION LIMIT	1	22		1	22		1	22		1	22	
BACKGROUND DEBERIS	Light			Light			Light			Light		
Cellulose Fiber	1	22		1	22		1	22		1	22	
Insect Fragments												
Plant Fragments												
Pollen												
OBSERVATION & COMMENTS												

- Same test conditions are pre.
- Quaduplicate spore trap tests. Very low. Shows we did not contaminate home as a result of the remediation.

Culture Testing Results.
Before & After. Both Very Low. But significantly lower after.



Pre Remediation Test Results



Post Remediation Test Results

Post Remediation DNA ERMI Air

- 6 hour air sampling.
- After cleaning the AC/ducting.
- After rebuilding the return air mixer box.
- After recleaning all surfaces and checking to make sure they were clean with the Swiffer test.
- After installing MERV 13 rated air filters in the returns and leaving the FAN=ON to clean the air of all mold spores, fragments, and by the way, ALL airborne MMIs.
- We performed 36 panel ERMI air testing (rather than 13 panel ARMI used for initial testing).
- Now nothing there.

LOCATION	Post Remediation 6hrs at 22 LPM
	Spore E./mg
Fungal ID / Sample ID	**#5**
Aspergillus flavus/oryzae	ND
Aspergillus fumigatus	2
Aspergillus niger	2
Aspergillus ochraceus	ND
Aspergillus penicillioides	2
Aspergillus restrictus*	ND
Aspergillus sclerotiorum	ND
Aspergillus sydowii	ND
Aspergillus unguis	ND
Aspergillus versicolor	ND
Aureobasidium pullulans	ND
Chaetomium globosum	ND
Cladosporium sphaerospermum	1
Eurotium (Asp.) amstelodami*	1
Paecilomyces variotii	ND
Penicillium brevicompactum	ND
Penicillium corylophilum	ND
Penicillium crustosum*	ND
Penicillium purpurogenum	ND
Penicillium spinulosum*	ND
Penicillium variabile	ND
Scopulariopsis brevicaulis/fusca	ND
Scopulariopsis chartarum	1
Stachybotrys chartarum	<1
Trichoderma viride*	ND
Wallemia sebi	1
Sum of the Logs (Group I):	**0.90**
Acremonium strictum	ND
Alternaria alternata	ND
Aspergillus ustus	<1
Cladosporium cladosporioides 1	4
Cladosporium cladosporioides 2	<1
Cladosporium herbarum	ND
Epicoccum nigrum	ND
Mucor amphibiorum*	ND
Penicillium chrysogenum	1
Rhizopus stolonifer	ND
Sum of the Logs (Group II):	**0.60**
ERMI (Group I - Group II):	**0.30**

Section Conclusions

- **If there is visible mold or continuing water. Fix.**
- **If there is surface dust, there is mold in the dust. Clean.**
- **If there is old carpet. Discard if a CIRS patient and replace with hard flooring. Otherwise clean.**
- **Put a MERV 13 or better filter in the AC or AC return.**
- **If you are still irritated in the home but not outside…**
- **If you are still irritated in the home the cause of the irritation is, by process of elimination, the HVAC System.**
- **That's where to focus your efforts.**
- **Not mold that does not represent exposure hidden in walls or attics.**

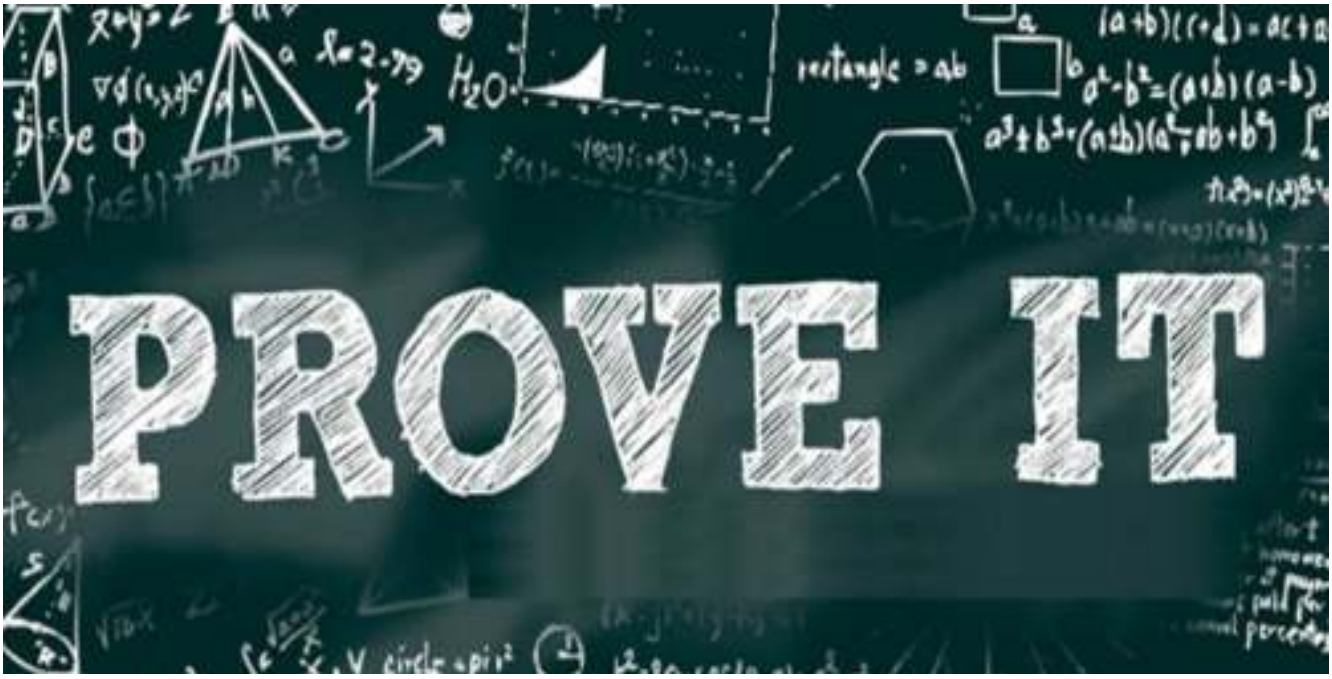
DOCTORS ARE NOT MOLD ASSESSORS

YOUR DOCTOR KNOWS ABSOLUTELY NOTHING ABOUT MOLD TESTING.

YOUR LIFE MAY DEPEND ON ACCEPTING THIS FACT.

TRUST YOUR DOCTOR BUT NOT ABOUT MOLD TESTING.

WE HAVE PROVEN



We Have Proven That Mold In Dust Does Not Represent Exposure

- DNA dust samples (HERTSMI-2 or ARMI/ERMI) **have nothing to do with mold in the air and therefore nothing to do with exposure.**
- Such dust testing should never be done.
- It is a waste of time and money and it overestimates exposure causing panic.
- If there is settled dust, clean the dust and then there will be no dust and therefore no mold in the dust.

Proof That Blood & Urine Testing Are Bogus

- The Robert B. blood readings as well as his urine testing show **erroneously** high levels of continuous exposure to toxic mold … because testing of R.B.'s air for mold and for mold fragments with DNA testing showed there is no current mold exposure and therefore no current mold toxin exposure.
- **If there is no current mold exposure and since the lab testing is said to be for current exposure to toxins … the lab testing is then proven bogus.**

Is It Mold or Something Else?

- According to Shoemaker SMCS, CIRS could be the result of any of the SMCS listed toxins/ inflammagens. (We call these Moisture-Induced Microbial Inflammagens — MMIs.)
- So why the focus on mold? No one appears to be interested in asking or answering this question.
- But the answer is that it can be easily tested for.

Range of toxins, inflammagens, and microbes found in WDBs		
Mycotoxins	Gram-negative bacteria	Hemolysins
Bioaerosols	Gram-positive bacteria	Proteinases
Cell fragments	Actinomycetes	Chitinases
Cell wall components	Nocardia	Siderophores
Hyphal framents	Mycobacteria	Microbial VOCs
Conidia	Protozoa	Building material VOCs
Beta Glucans	Chalamydia	Coarse particulates
Mannans	Mycoplasma	Fine particulates
Spirocyclic drimanes	Endotoxins	Ultafine particulates
Inorganic xenobiotics	Lipopoysaccharides	Nano-sized particulates

No Mold or Other Inflammagen Exposure

- However regardless of what the cause of CIRS (mold, mites, viruses, bacteria), if there are no active water leaks and the home and AC/ducting are clean/properly mold remediated and with a good quality (MERV 13 or better) air filter on the AC returns...
- And the humidity under control ...
- There will not only be no Mold exposure but also no other inflammagen (MMI) exposure.

CIRS Patients Can Start to Return to Health

- When there is no significant exposure to Mold or other MMIs …
- With proper medical treatment, CIRS patients that have been sick from mold toxin and/or other MMI contaminant exposure can start to return to health.
- When there are hidden contaminants that result in exposure, CIRS patients will not get better.

FINAL RECOMMENDATIONS PART 3

Use The Team Approach Focus on Visual Inspection

- CIRS patients: Hire a professional Team specializing in finding and fixing water damage including Mold Assessor (Indoor Environmental Professional), Mold Remediator, and AC contractor to thoroughly assess your home for sources of moisture/ excess humidity and of mold/mold fragment exposure.
- Perform a detailed visual, intrusive inspection including the HVAC system and if testing is desired, test the air for mold and mold fragments and not surface dust.
- But testing is hardly ever reliable for finding the extent, precise location and origin of mold for the purpose of identifying what and where to remediate.

Use The Team Approach & Focus on Visual Inspection

- Find and fix all moisture problems.
- The only area then that is constantly wet/moist but is a normal condition will be interior of the HVAC system.
- Can't do anything about that. But you can make sure that the HVAC interior including ducting is "as new" clean and then you eliminate all Mold/MMI exposure from the HVAC system.

Intrusive Inspections Required For CIRS Patients

- Again, the focus must be visual inspection for mold and water damage/stains. Seeing is believing.
- But focus remediation efforts only on actual sources of Mold/MMI exposure.
- Mold hidden in sealed walls or attics or crawl spaces that does not represent exposure…
- Do not remediate. Save your money.

For CIRS Patients. HVAC System Must Be Perfect

- For CIRS patients: You may wish to skip the HVAC System inspection and assume the HVAC system is not perfect.
- Refurbish HVAC to "as new" or replace especially the ducting with new.

Post Remediation DNA Air Sampling To Guarantee No Exposure

- After remediation and thorough cleaning of HVAC and all content, follow by post remediation air testing by DNA (ERMI or ARMI) sampling overnight.
- **Prove that there is no current exposure from either mold or mold fragments.**
- **Once the mold/fragment exposure is eliminated all other MMI exposure will also be eliminated.**
- **Then with proper treatment, CIRS patients that are sick from mold toxin and all other MMI exposure can start to get better.**

FREE Information

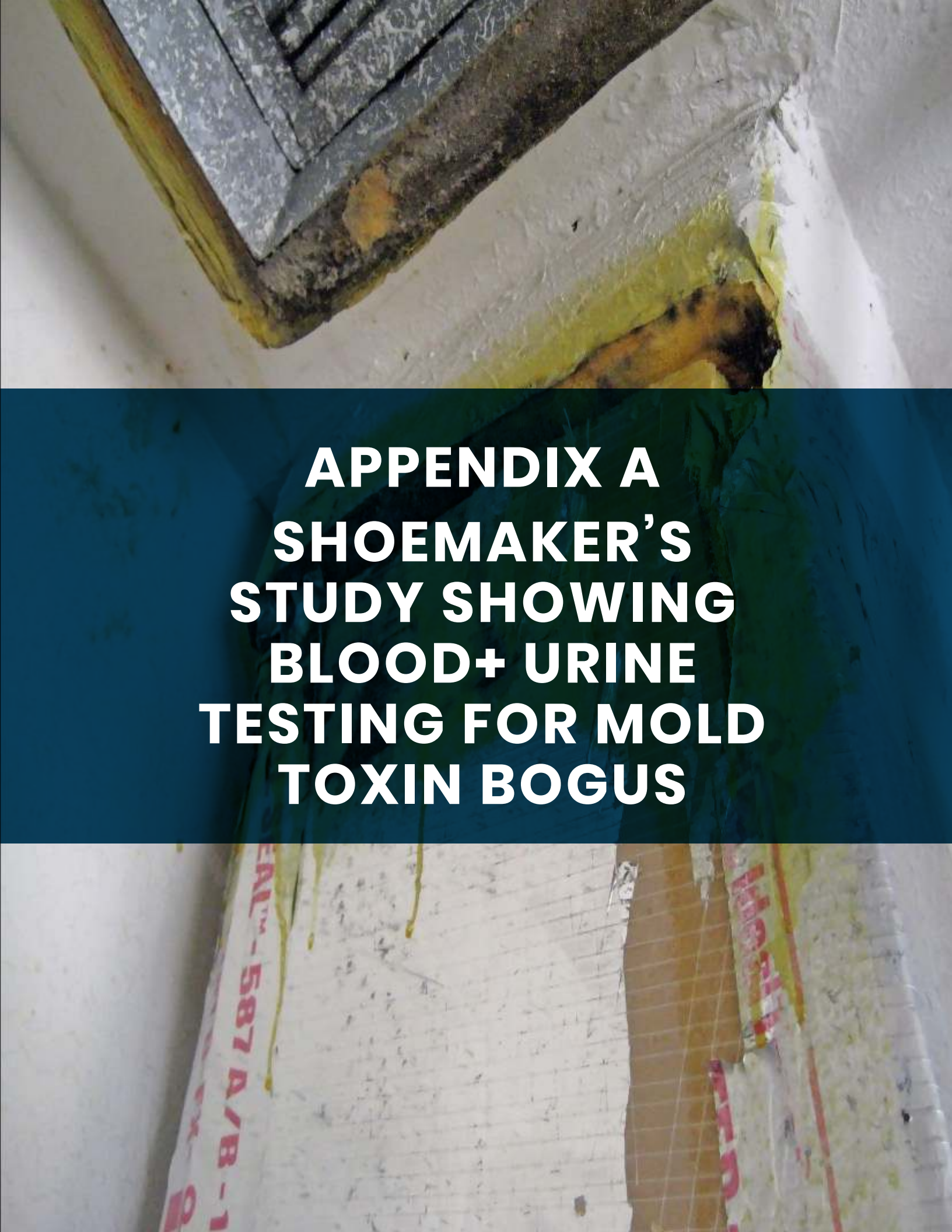
- For FREE information on mold assessment, water intrusion problems, and mold remediation see Dr. Rosen's:

www.Free-Mold-Training.org

For More FREE Information

- For more FREE information on Mold Toxins see Dr. Rosen's:

www.Mold-Toxins.com



APPENDIX A SHOEMAKER'S STUDY SHOWING BLOOD+ URINE TESTING FOR MOLD TOXIN BOGUS

Internal medicine review

Urinary Mycotoxins: A Review of Contaminated Buildings and Food in Search of a Biomarker Separating Sick Patients from Controls

October 2019

Urinary Mycotoxins: A Review of Contaminated Buildings and Food in Search of a Biomarker Separating Sick Patients from Controls

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List of acronyms:

CFS: Chronic Fatigue Syndrome
CIRS: chronic inflammatory response syndrome
CIRS-WDB: chronic inflammatory response syndrome acquired from exposure to WDB
CRS: chronic rhinosinusitis
DON: deoxynivalenol
GAO: US Government Accountability Office
GST: glutathione transferase
LPS: lipopolysaccharide
MARCoNS: multiply antibiotic resistant coagulase negative staphylococci

ABSTRACT

Beginning in 2010, there have been an increasing number of patients with a chronic multisystem illness who have been using measurements of mycotoxins in urine to diagnose a putative illness for which antifungals in various forms (oral, IV, sublingual and intranasal) are being used as therapy. Many of these patients and providers believe that the illness is caused by fungi living in the human body, making toxins, or has been acquired by exposure to the interior of waterdamaged buildings (WDB). This practice persists despite the absence
of (i) an accepted case definition; (ii) any validated control groups; (iii) any rigorous case/control studies; (iv) any prospective, placebocontrolled studies; (v) any confirmation of active fungal infection; (vi) any confirmation that urinary mycotoxins are not simply derived from diet; and presence (vii) of a sharp repudiation from the CDC of this practice and the use of antifungals in 2015; and presence (viii) of a robust literature demonstrating causation of illness acquired from WDB is inflammatory in causation, not infectious.

This review looks at the extensive published materials, including a definition of mycotoxins; a case definition for illness acquired following exposure to WDB from 2008 US GAO, multiple biomarkers, proteomics, transcriptomics, volumetric CNS imaging studies and more supporting the diagnosis of an active chronic inflammatory response syndrome (CIRS), acquired following exposure to the interior environment of WDB as a validated diagnosis that leads to use of published therapies with documented efficacy. The review also looks at (i) published literature of fungal contamination of foods; (ii) multiple world-wide studies showing contamination of urine with mycotoxins and metabolites in healthy controls finding 21 studies of 2756 controls with a range of positive urinary mycotoxins from 60 to 100%;

Keywords: CIRS, antifungals, mycotoxins, TGF beta-1, transcriptomics, ochratoxin A, deoxynivalenol, aflatoxin, trichothecenes, metabolites

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Background:
Attempts at definition: form or function?

There are multiple definitions for mycotoxins. Simple approaches such as secondary metabolites of fungi that can injure humans and other animals,omit some potentially pathogenic compounds or fungal elements, including beta glucans, hemolysins, mannans and spirocyclic drimanes. Defining mycotoxins as toxic substances made by fungi, would include other secondary metabolites such as antibiotics and immune suppressants. The main role for mycotoxins is to enhance efficiency of predation on plants,1 not acting as offensive or defensive functions, as is oft claimed.

These attempts at defining mycotoxins fail to address the problem of including a mechanism of injury or toxicity in vivo. Consideration of route of exposure such as ingestion of mycotoxins or skin exposure, versus inhalation brings about additional confounders. Assessing inhalation exposure injury stemming from exposure to mycotoxins in vitro ignores (i) protective host mechanisms, including antigen presentation; (ii) loss of regulatory control of immune responses; and in the case of waterdamaged buildings (WDB), (iii) eliminates the role of inflammatory responses, which taken as a whole, has been called chronic inflammator response syndrome (CIRS) since 2010. This syndrome is marked by innate immune activation following exposure to a diverse series of immunogenic effectors including over 30 published effectors found inside WDB.2

As we have seen 3, omitting consideration of differential gene activation following exposure to mycotoxins ignores the main mechanism of mycotoxin injury to people, namely ribotoxin and ribosomal inhibitory protein attack on ribosomal production (including initiation, elongation and termination) of protein 4. Further, failure to note suppression of nuclear encoded mitochondrial gene transcription is fatal to accurate assessment of adverse health effects.[3]

A more detailed definition of mycotoxins, but yet one that is still incomplete, focuses more on the role of mycotoxins in plants[5].

Mycotoxins are toxic secondary metabolic products of molds present on almost all agricultural commodities worldwide. Unlike primary metabolites (sugars, amino acids and other substances), secondary metabolites are not essential in the normal metabolic function of the fungus. Other known secondary metabolites are phytotoxins and antibiotics.

Currently there are around 400 mycotoxins reported. These compounds occur under natural conditions in feed as well as in food. Some of the most common mycotoxins include aflatoxins, trichothecenes, fumonisins, zearalenone, ochratoxin and ergot alkaloids. Mycotoxins are produced by different strains of fungi and each strain can produce more than one mycotoxin.

Each plant can be affected by more than one fungus and each fungus can produce more than one mycotoxin. Consequently, there is a high probability that many mycotoxins are present in one feed ingredient, thus increasing the chances of interaction between mycotoxins and the occurrence of synergistic effects,

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which are of great concern in livestock health and productivity. Synergistic effects occur when the combined effects of two mycotoxins are greater than individual effects of each toxin alone.

Given that mycotoxins in feed and food can be metabolized (in stomach, gut and liver) to make degradation daughters, we must expand our consideration of adverse effects of ingestion to include consumption of or endogenous production of metabolites of parent mycotoxins These compounds can stay in blood for variable amounts of time before appearing in urine, as enterohepatic recirculation can greatly reduce fecal excretion.6 Urinary excretion of mycotoxins and metabolites has become the main source of information regarding dietary exposure and metabolism, with a robust literature on findings of mycotoxins in urine of cases and controls.

The goal of this paper is to take the reader from assessment of a chronically ill patient, one exposed to the interior environment of a WDB before onset of illness, using a published protocol and to examine what benefit measuring mycotoxins in urine brings. We will review pertinent literature regarding mycotoxins in food; and mycotoxins found in urine in control populations to understand the firm stance of the Centers for Disease Control and Prevention (CDC) against use of urinary mycotoxin testing that leads to therapy based on antifungals.7 Recently published papers advocating treatment with antifungals based on urine findings will be reviewed[8,9]

Consider a functional definition of mycotoxins as different from what was presented above Mycotoxins are products of fungal metabolism in which secondary metabolites are manufactured in response to environmental stimuli that turn on mycotoxin synthetic gene clusters in the fungi, so they can make products that can be directly injurious to animals and people; and indirectly by adversely affecting protein production by impairing function of the sarcin-ricin loop in ribosomes and mitoribosomes; and mitochondrial function by interfering with nuclear encoded mitochondrial gene function.

We also cannot limit our discussion of naturally occurring mycotoxins as the mainsource of adverse human health effects. Fungi live in ecosystems in which a plethora of bacteria and actinomycetes invariably co exist.[10] Actinomycetes ("actinos") are adept at making compounds that are toxic; they can manufacture ribotoxins as well that co-occur with mycotoxins. Actinos are richly endowed with gene sequences to make a host of bioactive compounds, 11 including antibiotics, anti-virals, anti-parasites and immune suppressants, among others.

We need to separate out toxins made by actinos from endotoxins and fungal mycotoxins if we are going to impute adverse human health effects to mycotoxins. Assuming mycotoxins cause illness from simple exposure is untenable in the face of studies showing presence in healthy controls of urinary mycotoxins and their metabolites.

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Overview on dietary mycotoxins

For our discussion herein, we will be looking at three main categories of mycotoxins commonly ingested. Trichothecenes include some of the "dreaded" toxins made by some Stachybotrys spp. but they also are also produced by Aspergillus species.12 Trichothecenes are widely known, with types A and B toxins described. These compounds share unique structures that create a lack of specificity when measured using ELISA. Deoxynivalenol (DON) as a by-product of fungal gene activation, DON will induce production of peroxidases that block generation of hydrogen peroxide by a plant when the plant is being "eaten" by the Aspergillus species.1 The idea that fungi make mycotoxins as a defensive mechanism or as an offensive weapon to kill other fungi is not well supported.

The second category of mycotoxins of concern are ochratoxins. Ochratoxins have notoriety in the medical literature for their ability to cause renal injury, called Balkan nephropathy.

The third group of mycotoxins are aflatoxins Aflatoxins are made by several species of Aspergillus, especially A. flavus. Aflatoxins have a reputation for causing human health effects including liver damage and possibly cancer but when we discuss mycotoxins and prevention of mycotoxin injury following ingestion, it appears that in pigs, that supplementation of protein in the diet, to include glutamic acid in small amounts can prevent mycotoxin injury.

There are other toxins of importance to human health. When one thinks of trichothecenes one will hear of satratoxins and roridins. When one thinks of Wallemia sp. (and Aspergillus sp.) one will think of production of sterigmatocystin (STC). When we think of Chaetomium spp., it has its own suite of toxins, including chaetoglobosins.

Inside look: what adverse health effects are caused by exposure to the interior environment of WDB?

Over the last 25 years there have been a series of changes in opinions regarding causation of adverse health effects seen in patients exposed to the interior environment of WDB. Legal decisions mirror some views of society towards so-called "mold" cases.

Before it became known that mold illness was caused by inflammation, which was caused by exposure to WDB, followed by genomic response to biological elements found inside the WDB, including toxins, inflammagens and fragments of microbes (fungi, actinomycetes and bacteria, among others), some defense consultants suggested that the illness was allergy. There are people who do have allergies to mold; hypersensitivity pneumonitis can occur following exposure to thermophilic actinomycetes.

Allergy is based on excessive antibody responses to exposure; that finding is not involved in CIRS. Treatment of allergy by removal from exposure will resolve symptoms but not in CIRS. High levels of IgE is typically found in allergy but rarely is high IgE seen in CIRS. Defective antigen presentation is

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seen in CIRS; excessive antibody response is seen in allergy. Proteomic findings seen in CIRS but not seen in allergy include excessive levels of cytokines, split products of complement activation, TGF beta 1, with increased relative risk for a limited number of HLA haplotypes. CIRS is not allergy.

We now know that the genomic injuries in CIRS are commonly initiated by ribotoxins. These are compounds made by one-celled microbes, including fungi, bacteria and actinomycetes that stop or reduce normal protein synthesis by disrupting an evolutionarily conserved structural element on ribosomes called the sarcin-ricin loop (SRL). These are the actual players that initiate the cascades of inflammatory events seen in CIRS. The fact that the differential gene activation seen in CIRS comes from biowarfare among one-celled creatures that began four billion years ago is stunning.[13]

The legal importance of defining allergy as causative of symptoms acquired from damp buildings is straightforward: Negligent maintenance or construction defects don't cause allergy, but they do cause CIRS-WDB. Landlords might be accused causing injury to a tenant if the problem is CIRS but convincing a jury that the problem was allergy usually shields the landlord from paying awards.

Published research expanded our knowledge of harmful abnormal physiology of CIRS-WDB. We found that CIRS was definable. The condition had a variety of defining objective biomarkers. Findings that confirmed CIRS include visual contrast sensitivity (VCS), cluster analysis of symptoms, genetic susceptibility (HLA DR) and prospective repetitive re-exposure trials conducted over a course of six days (sequential activation of innate immune elements, SAIIE).[14-16]

While there are more science-based markers found in CIRS, nowhere will we see any that the illness is marked by presence of mycotoxins in urine. Presence of mycotoxins will tell us, however, if the patient has eaten warehoused foods in the last sixty days. The CDC has rejected such testing in no uncertain terms7, saying that foods have mycotoxins and that mycotoxins will appear in the urine of healthy persons following consumption of contaminated foods.

Additional insight into CIRS-WDB comes from multiple published, peer-reviewed case/control studies involving over 5000 patients. Patients have known exposures to WDB; controls do not. Cases have a large roster of symptoms, controls do not. Cases have lab abnormalities, controls do not Cases respond to treatment with reduction of symptoms, VCS deficits and lab abnormalities These elements form the accepted case definition of "mold illness" published by the US GAO in 2008.17 There is no mention of urinary mycotoxins in that Federal publication.

Prospective, double blinded, placebo-controlled clinical trials have also been published to confirm that causation of CIRS and treatment benefit is not random.[6, 18]

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Additional biomarkers come from NeuroQuant; an FDA-cleared software program added to MRI of the brain which shows a distinctive fingerprint in CIRS-WDB patients.[19] Correction of the inflammation that causes the illness results in simultaneous abatement of symptoms and NeuroQuant deficits, demonstrating that the neurologic and cognitive abnormalities in CIRS are not permanent.[20] The neurologic injury is caused by inflammation; it is treated by reduction of inflammation.

A subset of patients with CIRS-WDB have excessive gray matter nuclear atrophy that has been shown to respond to treatment protocols employing vasoactive intestinal polypeptide (VIP).2 Published in 2017, no other subsequent studies have shown correction of gray matter nuclear atrophy. Clinicians using CIRS treatment protocols see this salutary result on a daily basis.

Sequential mycotoxin testing has never been correlated with improvement of brain volumes.

There are other objective biomarkers in CIRS patients including development of pulmonary hypertension. Here, the velocity of tricuspid regurgitation (TR), measured in meters/per second, is elevated such that four times the square of TR, added to right atrial pressure, will exceed 30 as a cut off separating normal pulmonary artery pressure from acquired pulmonary hypertension. In a stress echo, measured by achievement of pulse rate of greater than 90% of predicted, we will see a rise greater than 8mm of mercury (Hg) in patients with acquired pulmonary hypertension.22 Sequential mycotoxin testing in urine does not correlate with improvement of pulmonary hypertension.

Measurement of maximal oxygen consumption exercise, VO2 max, can also be demonstrated in patients with CIRS-WDB. Correction of inflammation results in improvement of VO2 max. Sequential mycotoxin testing in urine does not correlate with improvement of VO2 max.

The greatest progress in looking at definable, objective biomarkers for CIRS23 and CIRSWDB comes from transcriptomics.3, 4 Using state of the art molecular platforms, transcriptomics shows differences in gene activity in cases compared to controls, as well as in patients observed prospectively who develop specific gene abnormalities with exposure to WDB that resolve with standard treatment. When combined with NeuroQuant studies showing resolution of gray matter nuclear atrophy and/or reduction of the enlarged forebrain parenchyma and/or cortical gray, these transcriptomic studies give us basis for better understanding of neuronal injury and repair. Sequential mycotoxin testing in urine does not correlate with improvement of transcriptomics.

A remarkable discovery of disproportionate increase in activation of coagulation genes in CIRS, together with beta tubulin genes, demonstrates the marked correlation of a subset of CIRS cases with enhanced gray matter nuclear atrophy. The significance of these findings for those with

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developing dementia supports the vascular hypothesis of neuronal injury,24-26 as targeted treatment of patients with excessive coagulation gene activation and early dementia is anecdotally associated with improvement of cognitive state.

Unfortunately, mycotoxicosis medical practice moves away from science

Against this deluge of overwhelming confirmation of causation of mold related CIRS illness and correction with published treatment protocols, one cannot forget discredited defense arguments presented in litigation. One of those opinions, called Geffcken, demanded that evidence of mycotoxins be found in patient tissues that were identical to mycotoxins found in a given building to which the affected patient was exposed. Unfortunately, the identification of mycotoxins accurately in a room is compromised even if mycotoxins could be found accurately in tissue. Geffcken held sway in medical mold cases until 2006 in the United States. Sequential assessment of urinary mycotoxins could have provided cover for Geffcken-type arguments in court had urinary measurements ever been shown in published literature to be correlated with exposure.

When Geffcken and allergy defenses didn't work to win cases, resourceful legal arguments from defense interests became that the illness seen in patients in water-damaged buildings was due to ingestion of mycotoxins. The idea of ingestion creating illness was supported largely by a study from Russia in 1947 reporting horses dying after eating hay contaminated by Stachybotrys sp. The study had little objective data to support it and yet, because it was a convenient way to make the jury look the other way, it was attempted without much success.

We still hear that ingestion remains the dominant source of exposure without evidence. Ingestion is the main route for appearance of mycotoxins in urine but remember metabolites (there are at least 18) of ochratoxin A can stay in human blood and tissue for over sixty days.[27]

Beginning in the mid-2000s, a new concept arose as advocated by several physicians from the Mayo Clinic. These physicians were convinced that fungi growing in sinuses were a marker for chronic rhinosinusitis (CRS) and that nasal cultures could be used to demonstrate the fungi.[28] Despite the evidence to the contrary,[29, 30] this idea, while it changed radically over the next five years from fungal causation of CRS in favor of an inflammatory condition with eosinophilic basic protein actually causing the runny nose, the idea has persisted. An important paper from the German literature showed that fungi could indeed be found in nearly every one's nose, with cases of CRS having 2.3 species of fungi, but controls had 3.1 species.[31]

Beginning in 2009, a new approach to explaining adverse health effects found in people exposed to water-damaged buildings was proposed. A pathologist published a paper[8] showing that patients exposed to WDB had putative evidence of mycotoxin carriage that could be detected using an ELISA technique in urine. Not with-standing the

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lack of precision of ELISA analyses, laboratories started selling tests for urinary mycotoxins. The idea that exposure alone equated to illness causation was promoted.

The paper referred to a control group but specific (i) control group demographics and (ii) building testing that confirmed microbial amplification were omitted. Absence of reliable control data remains a problem. If one is maintaining causation of illness, there needs to be prospective studies demonstrating acquisition of illness coincident with appearance of abnormal urine findings. If one relies on only a case/control study, there must be a transparent association in which abnormalities of exposed patients are different from abnormalities in non-exposed patients derived following transparent and thorough differential diagnosis.

The scientific concept is simple: no controls, no conclusions about cases.

Since the theory for finding mycotoxins in urine was that fungi growing in the nose were making toxins, use of antifungal nasal sprays rose in 2014 and 2015. By 2016, extensive acquisition of anti-fungal resistance was seen not just in fungi but in bacteria as well, apparently through the mechanism of horizonal gene transfer (personal communication, MicrobiologyDx, 8/2016).

The alarming feature of the new-found antibiotic resistance in bacteria was resistance to (i) vancomycin, an antibiotic necessary for dangerously ill septic patients, as well as (ii gentamicin, an aminoglycoside, emerged in a group of organisms called coagulase negative staph. These staphs are multiply antibiotic resistant and are known by their acronym of MARCoNS. Kirby-Bauer resistant biograms in MARCoNS let us trace development of vancomycin and gentamicin resistance which in turn could be traced back to physicians who used antifungals. Now that the antifungal resistance has spread (likely through plasmid exchange as well as free DNA transfer) the genes for fungal resistance are found not just in antifungal users but have spread rapidly in the MARCoNS population. MARCoNS are promiscuous exchangers of DNA and antibiotic resistance factors with other one-celled creatures. We simply need to look at the experience with Staph aureus, a coagulase positive staph, in the 1970's to 1980's to suspect that the reservoir of resistance to penicillin was in MARCoNS.

Dietary sources of mycotoxins

Fungi are ubiquitous in nature. Foods carry fungi. Moist food, especially starches, will support fungal growth in a few days. Dry foods will take longer to spoil, but fungal presence in foods can create problems for health of humans and animals. Predictably, toxigenic fungi are also found routinely in food supplies worldwide. In spite of the massive potential to cause adverse human health effects, i.e., if people eat fungi and mycotoxins at the same time, people will be ill and urinary mycotoxins will be positive, we may ask, Where are the cases? Absence of massive numbers of cases of mycotoxicosis suggests that the role of foods

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in producing chronic, multisystem, multisymptom human illness has not been confirmed. Yet, when we hear experts telling us to avoid coffee, mushrooms, wine, cheeses,breads and more because of fungi, as shown by mycotoxins in urine, we don't see any epidemiologic confirmation of the basis for such advice.

The source of mycotoxins in food can be divided into three categories of pre-harvest; post-harvest and warehouse-based growth. The foods involved are diverse, but colonization of foods is primarily due to several genera of fungi. These are Aspergillus, Fusarium and Penicillium, with Stachybotrys less common. Fusarium species are confined to corn products and will not be discussed.

In food manufacturing, from warehouse to table, attempts to destroy mycotoxins by food processing is an ongoing challenge. Mycotoxins are resistant to most physical methods; detection is complicated by the sheer volume of foodstuffs that must be analyzed.[32]

The health concerns about ingestion of mycotoxin-contaminated food have been heavily weighted to in vitro studies but confirmation of the same risks is rare in vivo. These problems could include autoimmunity, allergy, birth defects, cancers and mutagenesis.33 While the vast majority of patients seen following exposure to the interior environment of WDB have inhalation as their route of exposure, and not ingestion, in animals, ingestion can clearly cause mycotoxicosis. Species differences are dominant in mycotoxicosis.

There is variation from climate to climate of foods grown, as well as fungi found in those foods that contaminate harvested food. The most important mycotoxins are aflatoxin, ochratoxins, zearalenone, patulin and trichothecenes. This latter group includes deoxynivalenol (DON), metabolites of DON, T2 toxin and satratoxin.

Additional human health concerns include renal dysfunction due to ochratoxin A exposure, together with the largely uninvestigated field of chronic low-level, longterm exposure to multiple mycotoxins.[34]

The appearance of the most common fungi in food is at variance with fungi identified with confirmed adverse human health effects, measured with MSQPCR, called Health Effects Roster of Type Specific (formers) of Mycotoxins, version 2.35 For example, there are Aspergillus species that make ochratoxin found in food and WDB, but none of those fungi are seen in the top ten species list associated with adverse human health effects. Similarly, Stachybotrys, one of the top five species most pathogenic for human hosts, is associated with significant appearance of parent trichothecenes and their metabolites in urine of control patients. Interestingly, aflatoxin, which derives its name from Aspergillus flavus, a fungus that rarely appears in the top ten most commonly associated with human illness.

Patulin is a toxin found in fruit juices, especially in apple and grape juice, but also with stone

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fruits including apricots, peaches and plums. Patulin rarely is found in intact fruit but any fruit with a damaged surface is susceptible to fungal infestation. The key to preventing exposure to patulin is to maintain high fruit quality.

We are faced with the near ubiquitous finding of patulin in apple juice. But where is the evidence that when apple juice is ingested, bringing a significant gastric burden of patulin, that illness follows? Perhaps clues to the relative absence of adverse human health effects from ingested mycotoxins come from the findings that patulin, for example, is rapidly destroyed before leaving the stomach, resulting in a residual of less than 3%.[33]

Much of the concern about food contamination with mycotoxins has given rise to odd dietary alterations together with claims of enhanced safety of mycotoxin-free foods. As much as 50% of human daily intake of ochratoxin and its metabolites is due to its direct consumption in cereals or grains, but the remainder will be due to consumption of animals after they ate contaminated feed. The list of common food sources of ochratoxins includes foods made from corn, rice, wheat, barley, oats, rye, sorghum and millet. Contaminated foods include cereals or trail mixes; together with bread, bread products and baby food. Any brans from rice, corn, wheat or oats can be contaminated as well as cracked grains, wine and beer.

Ochratoxin A is found in cheese and meat products, as well as dried and smoked fish, soybeans, garbanzo beans, nuts and dried fruits. Additional food sources of ochratoxin are raisins, wine and wine vinegars. Coffee and pork also are known to harbor ochratoxins.[33]

The problem of analysis for ochratoxin is complex, usually requiring high performance liquid chromatography (HPLC) and mass spectrometry (MS) in an attempt to separate an apparent molecule of ochratoxin A from its 18 known congeners that have variable half-life in blood, ranging up to 60 days.33 With so many degradation daughters of ochratoxin A, where are the billions of people suffering worldwide from ochratoxin A-associated nephropathy? The stated risks of OTA appear to be overstated. With regard to cancer causation, aflatoxin (AFB1), especially when associated with hepatitis B virus, is widely reported to be associated with hepatic cell carcinoma. The marker for the breakdown product of aflatoxin is AFB1-N7-guanine adduct.32 This adduct is secreted into urine; mycotoxins labs could look for this marker of cancer causation from aflatoxins. One might again ask, where are the billions of people with cancer associated with aflatoxin?

Host/farm protective factors

It is well known that detoxification of aflatoxins is accomplished internally by an enzyme, glutathione S-transferase (GST), which will bind to an ingested metabolite of aflatoxin, then combine with glutathione to detoxify the compound. GST is ancient and evolutionarily conserved, with a complex gene family in plants.12 T2 toxin and DON

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will induce activation of groups of the GST genome. Of interest is the existence of gene super-families for GST in honeybees and Drosophila;36 polymorphisms are felt to have a role in loss of protection from sporadic colorectal cancer risk in Caucasians.[37]

Regarding sources of T-2 toxins in the world, the natural occurrence of host genera Fusarium or Sporotrichioides has been reported in Asia, Africa, South America, Europe and in North America. Predominant genera that make other trichothecenes include Stachybotrys, Trichoderma and Trichothecium. All of these sources of T-2 will be detected when an ELISA assay is used.33 The main production of mycotoxins is associated with the greatest water content before and immediately after harvest. Once food materials dry out, mycotoxin production declines in step with reduction of Aw.

DON and its metabolites are the most prevalent of trichothecenes found in food; DON is found with its metabolites, together with T-2 toxin and nivalenol. DON in urine is easily separated from other trichothecenes. Only a paucity of commercial labs, however, will perform this standard assay. Given the disparity of known effects of trichothecenes compared to known adverse ribotoxin effects creating the fundamental mechanism of molecular hypometabolism with ribosomal break down in mammalian cells following exposure, more accurate delineation of trichothecene effects is needed.

A problem faced by microbiologists is how to separate direct ribosomal injury from DON versus indirect injury from ribotoxins or ribosomal inhibitory proteins. This problem of lack of specificity as part of DON toxicity is partially solved by identifying a ribotoxic stress response that is manifested by immunotoxicity causing enhanced activation of mitogenactivated protein kinases (MAPK), which can be used as a marker for exposure to trichothecenes.

The association of enhanced mycotoxin production with activity of water (Aw) bears consideration. While there are variable activities of water associated with growth of fungal species, production of mycotoxins is most commonly accomplished at a higher Aw of 0.98 for ochratoxins, 0.93 for fumonisins and 0.90 for DON noted.33 These values of Aw are not found on mucus membranes in humans, especially the nose. These data rule out the possibility of intra-nasal production of mycotoxins.

Given the common finding of mycotoxins in food and the common finding of mycotoxins in urine, what can we decide about the absence of significant health effects of the billions of patients exposed to mycotoxins on a daily basis? Could there be dietary factors associated with protection from mycotoxicosis? Are we looking at enhanced metabolism? Are we looking at the effect of antioxidants? Glutathione? Perhaps we can learn from mammals that are far more sensitive to mycotoxin effects, as shown by reduction in growth rates, than others. Pigs lead the list. As mentioned, pork will have mycotoxins

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in it and while the diet of a factory raised feedlot swine operation is not typified by feeding animals slop, mycotoxins, especially DON, in pig food can slow growth causing the grower significant financial loss.

Three papers from the Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South Central China38-40 bear significant weight in assessment for host factors protecting piglets from ingested mycotoxin injury. Multiple groups of piglets were assessed for DON-damage by measurements of oxidative parameters in the first study38 including catalase, malic dialdehyde, nitric oxide, peroxide levels in blood; total antioxidant capacity; d-lactate and amino acids. DON decreased catalase but this effect was blocked by feed that was supplemented by 2% glutamic acid. Peroxide too was higher in DON, but glutamic acid blocked that oxidative effect as well. Similar results were noted for malic dialdehyde and nitric acid: glutamic acid prevented harm. DON increased lactate (NB: this is an indirect marker for molecular hypometabolism) but glutamic acid prevented that abnormality.

Villus height was diminished in jejunum and ileum by DON but was increased in glutamic acid-fed piglets. Similarly, glutamic acid blocked increased lymphocytes induced by DON, and protected goblet cells as well. The indicator genes for DON-induced ribotoxic stress response were activated in DON-fed pigs and protein synthesis reduced, but not in pigs fed the combination of DON and 2% glutamic acid.

The second study[39] used the same control and experimental designs. This time, glutamic acid blocked mycotoxin-induced decreased weight gain and blocked reduced feed conversion rate. The final study40 used nuclear magnetic resonance to show additional benefits of glutamic acid in DON-challenged piglets. Here the authors showed additional manifestations of protection, namely glutamic acid treatment corrected DON-driven raised levels of LDL cholesterol, lowered levels of HDL; corrected elevated levels of alanine, arginine, acetate, glycoprotein, trimethylamine-N-oxide, glycine, lactate, urea and glutamate/creatinine ratio. Further, glutamic acid increased superoxide dismutase and glutathione peroxidase. The authors conclude that glutamic acid can repair injuries associated with oxidative stress as well as disturbances of energy and amino acid metabolism induced by DON.

Remember that glutamate, consists of two enantiomers. One is L-glutamate and the other is D-glutamate. The D-enantiomer of glutamate is better known as MSG. Glutamate is found in proteins and peptides, with virtually every food containing glutamate. If we superimpose the occurrence of aflatoxicosis on areas of protein/calorie malnutrition, we see that if adequate protein in food is ingested, mycotoxicosis from food is dramatically reduced. This is an association suggesting that something found in food has much to do with protection from fungal injury. Protein rich foods including meat, eggs, poultry, milk, cheese and fish are major components of glutamate in the diet.

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The 3-D structure of glutamate has an amino group in the middle of the chain of five carbons with a carboxylic acid moiety found on either end. At acid pH, one hydrogen can be lost from a hydroxyl group balanced as a zwitterion with a NH3 replacing an NH2. At gastric pH, there can be production of a single positive charge with the amino group becoming an ammonium group with each hydroxyl group losing an electron. This mini-molecular anion dipole could create a mechanism for binding the anion rings of mycotoxins by the cation found in glutamate at gastric pH. This is the putative mechanism that has been shown for cholestyramine binding to a variety of mycotoxins.[41, 42]

As at least 25% of foods are felt to be contaminated worldwide, we would expect anywhere between 1 ½ to 2 billion individuals sickened by mycotoxins if this were a simple linear expression of causation with exposure resulting in illness. The absence of 2 billion sick people suggests strongly the model used for excessive mycotoxin pathogenicity is flawed. Even though food safety concerns and best farming practices emphasize mechanisms to reduce fungal contamination, we need to look at host factors as the variable controlling acquisition of illness from ingestion.

Host factor analysis itself is potentially flawed as rarely mentioned fungi may be confounders. Such is the case with sterigmatocystin (STC) in foodstuffs. While we worry about production indoors of STC by Wallemia sebi, STC is also found in grains, corn, bread, cheese, spices, beans, soybeans, pistachios, animal feed and silage.43

In a Turkish population,44 hydroxydeoxyguanosine and malondialdehyde were correlated with ochratoxin A. The advantage of finding specific urinary markers with mass spectrometry is that there are 18 different metabolites known for ochratoxin A Trying to pick one of those 18 accurately as a carcinogen by any other method is problematic.

Dietary protection factors

The degradation of patulin showed 94% disappearance from blood within 2 minutes of ingestion.45 Given the high concentrations of patulin in particular foods, especially fruits, with apples leading the way, these compounds are rapidly degraded before reaching other tissues inside the upper gastrointestinal tract.

Further confirmation of the disintegration of patulin comes from studies in rats.46 Isolated rat stomachs had luminal application of patulin with rapid emergence of mycotoxin into the stomach. Concentrations of 350 mg per liter and 3.5 mgs per liter were tested, with mycotoxins appearing almost instantly with both the high and the low dose. Residual toxin was 3% and 0.06%, respectively, in gastric tissue. This disintegration of 8400 micrograms and 700 micrograms, respectively, was in part due to the role of intracellular glutathione (GSH). The massive dose of patulin did reduce GSH content of tissue by 87% of controls but not the low dose.

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Dietary supplementation of animal feed with organic activated bentonite, a clay product, and humic acid polymer, both have shown benefit in vitro,47 confirmed by binding by both bentonite and humic acids of ochratoxin and zearalenone, with binding exceeding 96% of total burden. These products have not been demonstrated to provide benefit of reduction in inflammatory biomarkers acquired following exposure to WDB. Given the ability of these compounds to adsorb toxins but not to prevent disease suggests that ingestion and gastrointestinal exposure to mycotoxins is not the relevant causative feature of CIRS-WDB.

Other dietary strategies48 with the ability to prevent toxic effects of ingested mycotoxins include antioxidants (selenium, vitamins, provitamins); food components, including fructose, aspartame, chlorophyll and phenols; together with biological binding agents, hydrated sodium, calcium, aluminosilicate, bentonites, zeolites, activated carbons; bacteria and yeast. While these dietary strategies provide promise, the discrepancy between in vitro studies and in vivo studies are difficult to reconcile. These additional dietary compounds just don't work to prevent CIRS-WDB; the problem is not ingested mycotoxins.

Additional efforts to use microbiologicals for deactivating mycotoxins show initial promise. A Eubacterium sp., (BBSH 797), isolated fromthe rumen of a cow, deactivated trichothecenes. Also, a novel yeast strain, Trichosporonmycotoxinivorans was isolated and characterized as being able to degrade ochratoxin A and zearalenone.49

In a study that shows great promise,50 32 separate strains of Rhodococcus were demonstrated to be able to degrade aflatoxin B- 1, zearalenone, fumonisins B-1, T-2 toxin and ochratoxin A. In addition, Rhodococcus species were able to protect from injury from multimycotoxin exposure. While this was a promising study in 2013, no Rhodococcus strains are available commercially.

Other microbiologic interventions include mycotoxin-degrading bacteria and fungi isolated from agricultural soils and animal digestive tracts. 51 Biotransformation effects included acetylation, glycosylation, ring cleavage, hydrolysis, deamination and decarboxylation. These promising solutions have not been tested in humans yet.

Similarly, Trichosporon shows promise in its ability to detoxify ochratoxin A. Certainly, this organism could be used in clinical trials to deactivate mycotoxins in animal feeds. This yeast, isolated from a hind gut of the termite,.52 Mastotermes darwiniensis, shows promise.

Enzymatic inactivation (biological detoxification) of fungal toxins has been accomplished using pure cultures of bacteria and fungi.53 Following isolation of a complex microbial population, after differential gene activation has been identified, cloned and expressed in other hosts, the ability to detoxify aflatoxins, cercosporin, fumonisins, fusaric acid, ochratoxin A, oxalic acid, patulin, trichothecenes and zearalenone was accomplished.

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Regarding glutathione,54 in tests performed in lambs, there was localization of injected aflatoxin found in liver, nasal olfactory mucosa, nasopharynx, esophagus. larynx, trachea, bronchi, bronchial and conjunctiva. The nasal mucosa was the most active in forming DNA bound aflatoxin metabolites. When incubated in the presence of reduced glutathione, a drastic decrease in active DNA binding was seen without the addition of GST.

Experiments done on chickens fed ochratoxin A[55] provided yet an additional approach of prevention of mycotoxin-induced injury in animals. When chickens were fed ochratoxincontaminated diets of up 1.5mg/kg for three weeks, there was marked reduction of relative weight of immune organs (bursa of Fabricius and spleen).

Phagocytic function and lymphoproliferative responses in a follow-up experiment, when chickens were treated with silymarin, vitamin E or antioxidants,56 the ochratoxin-induced immunotoxicity was prevented. This approach shows promise for chickens but as yet there is no indication of benefit in humans.

The mechanism of DON-induced proinflammatory gene expression57 appears in humans and animals to involve activation of protein kinases located on the damaged ribosome. DON-induced activation of mitogenactivated protein kinases (MAPKs) is the known ribotoxic stress response. Pathological abnormalities in chronic low dose exposure showed anorexia, impaired weight gain and growth hormone dysregulation together with high dose exposure-evoked gastroenteritis, vomiting and a shock-like syndrome. DON evokes a ribotoxin stress response in mononuclear phagocytes which is an important contribution to acute and chronic toxic effects in vivo.

Early work in this field from Pestka58 has shown that the mechanism of ribotoxic stress response involves double stranded RNA activated protein kinase (PKR) as well as hematopoietic cell kinases (Hck). Inhibitors in gene silencing studies have revealed PKR plays roles in both DON-induced gene expression and apoptosis.

Pestka has investigated the role of trichothecenes59 on white blood cells. His lab has found that monocytes, macrophages as well as T- and B-lymphocytes are cellular targets of DON and trichothecenes. Exposure, even to low dose concentrations, reflected upregulation both transcriptionally and post-transcriptionally of cytokines, chemokines and inflammatory genes. High concentrations of exposure bring about apoptosis of leukocytes. Again, Pestka discusses the ribotoxic stress response, with binding to ribosomes and rapidly activating MAPKs. We are seeing that the series of immune events in CIRS-WDB is not related to ingestion but is related to genomic and transcriptomic abnormalities induced by toxin and/or ribotoxin exposure.

Experiments with DON inoculation60 showed a whole series of gene activation in the MAPK family. For example, tyrosine phosphorylation of the hematopoietic cell kinase, Hck, was detected within 1 to 5 minutes after addition of toxin, with this gene activation suppressed by incubation

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with inhibitors of the family of tyrosine kinase. Investigating the source of apoptosis has shown that BAK, a pro-apoptotic Bcl-2 family protein, is expressed in a wide variety of tissues.61 Bcl-2 proteins regulate apoptosis as well as autophagy. When activation of apoptosis occurred following treatment with nigericin, a ribosomal toxin, both transient and stable overexpression of various forms of BAK exerted a protective role but it did not inhibit the extent of nigericin-mediated activation of caspase-3. This study strengthens the link between an exposure to ribotoxins and induction of apoptosis.

Accurate mycotoxin analysis in urine, feed and food: GC, MS and others Atutorial

Mycotoxins are toxic fungal secondary metabolites that frequently contaminate food and feed worldwide, and hence represent a major hazard for food and feed safety. To estimate human exposure arising from contaminated food, so-called biomarker approaches have been developed as a complementary biomonitoring tool besides traditional food analysis. [62-73, 110] The first methods based on radio-immunoassays and enzyme-linked immunosorbent assays as well as on liquid chromatography were developed in the late 1980s and early 1990s for the carcinogenic aflatoxins. In the last two decades further tailor-made methods for some major mycotoxins have been published.

Since 2010, there has been a clear trend towards the development and application of multianalyte methods based on liquid chromatography/electrospray ionization/tandem mass spectrometry for assessment of mycotoxin exposure made possible by the increased sensitivity and selectivity of modern mass spectrometry instrumentation and sophisticated sample clean-up approaches. With use of these advanced methods, traces of mycotoxins and relevant breakdown and conjugation products can be quantified simultaneously in human urine as so-called biomarkers and can be used to precisely describe the real exposure, toxicokinetics, and bioavailability of the toxins present.

In this article, we present a short overview of the above cited articles and a comparison of published multi-biomarker methods focusing on the determination of mycotoxins and relevant excretion products in human urine is presented. Special attention is paid to the main challenges when analyzing these toxic food contaminants in urine, i.e., very low analyte concentrations, appropriate sample preparation, matrix effects, and a lack of authentic, NMRconfirmed calibrants and reference materials. Finally, the progress in human exposure assessment studies facilitated by these analytical methods is described and an outlook on probable developments and possibilities is presented.

Traditionally, mycotoxin testing used enzyme linked immuno-sorbent analysis (ELISA) technology which relies on antibodies, sometimes monoclonal but more often polyclonal. Among all published

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immunological based methods, these enzyme-linked immunosorbent assay (ELISA) were the most commonly used for mycotoxin determination. ELISA provides rapid screening, with many kits commercially available for detection and quantification of major mycotoxins including AFs, AFM1, OTA, ZEA, DON, fumonisins, and T-2 toxin.

ELISA methods have been validated in a wide variety of food matrices by only in a few instances for urine. The principle of ELISA is based on the competitive interactions between mycotoxins (acting as an antigen) and assigned antibodies labelled with toxin-enzyme conjugate for many binding sites. The amount of antibody-bound toxin-enzyme conjugate will determine the level of color development. This technique provides a rapid, specific, and relatively simple method for analysis of mycotoxins.

However, ELISA has certain disadvantages including potential cross-reactivity, dependent on antibody specificity. In addition, the kit detects only a single mycotoxin and is designed for one-time use; thus, it can be costly, impracticable even, if one needs to test samples contaminated with multiple mycotoxins. Moreover, each test kit is specified by the manufacturer for a set matrix and while some third-party validations, e.g., by AOAC, have been done for some mycotoxin ELISA kits, the validations are for use with specific toxins under specific contamination levels within specified matrixes; therefore, the kit cannot be used for all food matrices and all contamination levels, let alone human samples like blood and urine. Even when used in their appropriate settings, the manufacturers of these kits recommend that positive ELISA results should be confirmed by a suitable chromato-graphic method, especially when used in a matrix not specified by the manufacturer.

Alternatively, lateral flow devices (LFD) has been developed as a single-step test that includes a negative control line along with the sample lines on the same strip. A lateral flow test can provide semi-quantitative results in less than 10 min and requires no specialized equipment. It consists of three parts: a conjugate pad, a porous membrane, and an absorbent pad. The test is based on a competitive immuno-assay, where a labelled antibody is used as a signal reagent. This device has also recently been coupled with spectrometric readers to provide quantitative results. LFDs are commercially available for detection of AFs, DON, T-2 toxin, OTA, and ZEA. However, their applications in the field is limited due to numerous problems associated with the sensitivity and reliability in different matrices in addition to their high cost.

Another simplified system comprises flowthrough membranes which utilize the same basic principle as LFD but may not yield accurate results near the detection limit. Flowthrough immunoassays have been developed for screening OTA in green and roasted coffee, AFB1 in nuts and ZEA in cereals and feed samples. Although many different rapid strip tests have been developed for detection of major mycotoxins in different food commodities, they are not commonly used in the field and not commercially successful due to problems related to sensitivity, cost, and accuracy.

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In addition to the methods described above, several other research methods have potential utility for the analysis of mycotoxins. However, these methods have limited application and have not been widely used outside the research environment as they require further verification and validation by recognized bodies such as AOAC, International Organization for Standardization (ISO) or CEN. There are commercially available test kits which are ready-to-use lateral flow devices (LFD) designed for on-site testing, providing rapid analysis of a wide range of food and feed samples with an assay time of 3 minutes. The test kits are available (62) in a qualitative or quantitative format, which requires a reader to provide objective results and secure a consistent results documentation.

Determination of mycotoxin levels in food samples is usually accomplished by methods (63) that include certain common steps: sampling, homogenization, extraction followed by a clean-up, and finally the detection and quantitation which is performed by many instrumental and non-instrumental techniques.

Chromatography is the most commonly used method used for mycotoxin analysis in food and feed. The earliest chromatographic method was thin layer chromatography (TLC), which is presently still used as a rapid screening method for certain mycotoxins by visual assessment or instrumental densitometry. However, current trends in mycotoxin analysis in food are focused on application of robust, fast, easy to use, and cheap technologies that are able to detect and quantify various mycotoxins with a high sensitivity and selectivity in a single run. To meet those needs, many chromatographic methods such as high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV), diode array (DAD), fluorescence (FLD), or mass spectrometry (MS) detectors and UHPLC or UPLC with reduced column packing material have been developed. Additionally, gas chromatography (GC) coupled with electron capture (ECD), flame ionization (FID), or MS detectors have been used to identify and quantitate volatile mycotoxins. Due to the low volatility and high polarity of most mycotoxins, GC analysis often requires a derivatization step; therefore, this method is used rarely in mycotoxins analysis which has been greatly advanced by coupling liquid chromatography techniques to massspectrometry (e.g., LC-MS; LC-MS/MS).

Apart from the great advantages of the conventional HPLC methods mentioned above, MS offers several distinct advantages over all LC methods for mycotoxin analysis in food. Basically, the mass spectrometer works by ionizing the molecules, and sorts and identifies= them based on their mass-to-charge ratio (m/z). MS offers higher sensitivity and selectivity, as well as chemical structural information by molecular identity of the analyte based on m/z providing the mass spectrum as an ideal confirmatory technique. MS detection reduces time by eliminating the need for error-prone sample derivatization and clean-up steps needed for fluorescence enhancement. Different MS interfaces and analyzers have been used, such as atmospheric pressure chemical ionization (APCI),

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electrospray ionization (ESI), and atmospheric pressure photoionization (APPI). In addition, there are many types of mass analyzers such as quadrupole, time-of-flight (TOF), ion-trap, and Fourier transform-ion cyclotron resonance (FTICR). ESI, triple quadrupole, and TOF have been used extensively for mycotoxin analysis. Although the early applications of MS were for the analysis of single mycotoxins, the technique can now simultaneously quantify many hundreds mycotoxins and their metabolites in a single run, making it the current method of choice for detecting multiple mycotoxins in a wide variety of foods.

Since the arrival of modern high-performance LC-MS/MS (liquid chromatography-tandem mass spectroscopy) and GC-MS/MS (gas chromatography-tandem mass spectroscopy) instruments enabling multi-analyte methods for mycotoxin determination have become available, however these are not without substantial cost, with the majority of mycotoxin determination in urine performed recently by LC-MS/MS . However, a major challenge in urine mycotoxin analysis is the extremely low analyte concentrations present following dietary exposure.

Thus, effective, specific, sensitive and accurate methods for mycotoxin detection in urine require appropriate sample preparation protocols to accomplish the desired sensitivity while obtaining acceptable limits of detection (LODs) and quantitation (LOQs). Most of the methods available in the literature are based on traditional extraction techniques such as liquid/liquid extraction (LLE) or solid/liquid extraction (SLE), which have several disadvantages, mainly the high solvent volumes, high amounts of sample, and the long times required for the analysis. In recent years, method simplification and miniaturization are the most important trend in sample preparation allowing the use of low sample and solvent volume, fast analysis, and greater efficiency. Many pages of this monograph could be devoted to discussion relevant to the best method of prep-aration of urine for analysis, while other laboratories use the same dilute and shoot method for urines, as they do for bulk samples, blood and dust. In this case, a broad spectrum of spiked control mycotoxins is necessary to confirm matrix effects are being avoided and to enable the analysis to be shown to be linear, despite interferences and inhibition by such compounds that are often found in urine.

Method validation by laboratories undertaking mycotoxin analysis should follow the guidelines established by the EU and other regulatory bodies, including the determination of linearity, matrix effect (ME), limits of detection (LODs), limits of quantitation (LOQs), recoveries, repeatability (intra-day precision), and reproducibility (inter-day precision).

Of utmost importance are calibration curves for all mycotoxins analysed – they must be constructed from standard solutions (external calibrators) and in the matrix (matrix-matched calibrations). Matrix-matched calibration curves should be prepared from blank urine samples spiked with standardised mycotoxins before and after extraction, if used. There are limits for the range

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of these standard curves that should also be observed so that LOQ is also validated.

A final, cautionary note about validating analytical methods for mycotoxins is that reliance on avoidance of foods likely to contain mycotoxins or their metabolites is no guarantee that the urine obtained from control subjects will be a genuine baseline. Screening and analysis of the analytes of interest is therefore prudent because mycotoxin producing moulds may contaminate numerous agricultural commodities either before harvest or during storage. A varied diet consisting of different foods may therefore be contaminated with a range of mycotoxins.

It is claimed that naturally occurring mycotoxins have been indicated as causative of a wide array of adverse health effects. The measurement of urinary mycotoxin levels is a means of assessing an individual's exposure, but the development of sensitive and accurate analytical methods for detecting mycotoxins and their metabolites in urine samples is challenging. Urinary mycotoxins are present in low pg/ml concentrations, and the chromatographic identification of their metabolites can be obscured by other endogenous metabolites.

As a result of the advent of the latest generation of high-performance LC-MS/MS instruments, a clear trend towards the development and application of multianalyte methods in mycotoxin biomarker research can be observed. Purification of the analytes is often achieved by using sophisticated sample cleanup approaches with subsequent separation by liquid chromatography and detection using triple-quadrupole analyzers coupled via an electrospray ionization (ESI) interface. However, the latest studies have also successfully applied the so-called dilute and shoot approach by omitting any clean-up step. As already stated, this section only provides a short overview and comparison of published multi-biomarker methods, and is expanded further to discuss challenges associated with very low analyte concentrations, sample preparation, matrix effects and a lack of calibrants and certified reference materials, and describes the progress in human exposure assessment studies facilitated by these methods in following sections.

A major challenge in mycotoxin biomarker research are the extremely low analyte concentrations present in biological fluids following dietary exposure. Hence, appropriate sample preparation protocols are crucial to obtain acceptable LODs. This is, however, hampered by the great chemical diversity of analytes typically included in multi-biomarker methods. This issue becomes even more complex once polar conjugates such as glucuronides are included as they are frequently lost during common clean-up approaches such as SPE or IAC procedures.

Accurate mycotoxin analysis in urine, feed and food: GC, MS and others From the literature

The State-of-the-Art method74 for testing mycotoxins including aflatoxin, ochratoxin and trichothecenes is mass spectrometry. Gas chromatography (GC) and liquid chromatography (LC), liquid mechanisms, while regarded as

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accurate and precise, may show extreme variable sensitivity due to different biological characteristics of mycotoxins, matrices and instruments. This is especially true for LC-MS where the response can be different depending on ionization techniques used. If fluorescence or UV absorbance can be used for quantitative measurements LC-MS appears to be relegated to use as a confirmatory technique.

If toxins are not volatile, LC-MS is uniquely able to render results that are quantitative and qualitative accurately. These problems are multiplied by attempts at determination of mycotoxins in food given the extreme variability of the food matrices. Specific ionization interfaces are needed to reduce matrix effects and ion suppression. It is possible that MS detectors will show advances that permit low cost, high throughput determination of mycotoxins in food and feed.

Given the concerns about health effects of mycotoxins in food and feed, risk assessment of mycotoxin contamination for both humans and animals require clear identification and reliable quantitation in diversified matrices.75 With mass spectrometry emerging in the 1970's, we now are seeing a variety of hyphenated techniques that combine chromatography with mass spectrometry. Indeed, LC-MS, or better still LC-MS/MS has become a routine technique.

The challenge of detecting multiple mycotoxins, as is commonly seen in the same sample, requires advanced techniques for each diagnostic run. LC-MS/MS is able to measure different levels of mycotoxins, and their metabolites, that are both free and masked. Newer techniques will likely emerge as multidimensional chromatography-MS, capillary electrophoresis-MS and surface plasmon resonance array-MS have become available. Cost of the new advanced techniques will continue to be a factor.

With the enhanced multi-class mycotoxin analysis in food, environmental and biological matrices and LC-MS/MS, the ability to detect mycotoxins has become increasingly precise. This technical advance raises a curious condition, however, in that presence or absence of molds are less frequently identified and correlated with mycotoxin presence.76 As mentioned, mycotoxins seldom develop alone; various types will be formed in the same foodstuff. Co-occurrence of mycotoxins creates a real problem for assessment of dose-response relationships, not to mention genetic susceptibility such that the mere presence of multiple mycotoxins should be considered as a risk factor but risk itself is not adequate to conclude causation of illness.

A simple question for governing bodies regarding food safety is how one device can provide results that will be sensitive and specific for the wide variety of chemical structures in mycotoxin analysis.77 An additional challenge remains that heterogeneity of foods demand multiple analytical methods be used at the same time permitting rapid and inexpensive analysis. Ongoing problems include proper collection of representative samples, avoiding secondary contamination after collection, performance of emerging

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analytical methods, including immunochemical techniques, with validation of methods for those involved with enforcement of standards in regulatory affairs and finally, limitation of current methods.

In an effort to confirm precision of increasingly sophisticated mass spectrometry techniques, a study was done by Gerding in Germany.78 Food surveys were recorded together with the food frequencies questionnaire followed by LCMS/MS assessment of urinary biomarkers. The authors looked for 23 separate urinary biomarkers, including trichothecenes (especially DON and its metabolites), T-2 toxin, HT-2 toxin, aflatoxins and aflatoxin metabolites, ochratoxin A, ochratoxin alpha and others. One or more of a group of six mycotoxins and urinary metabolites were detected in 87% of the samples in a single occurrence. Only DON and its metabolites were detectable in quantifiable amounts. No statistical significance for correlation of staple food intake with urinary biomarker concentration could be provided. This important study supports the commonality of daily exposure of healthy patients to mycotoxins with such mycotoxins being identifiable in urine. The study could not control for a variety of metabolic modifications together with metabolites to provide statistical surety of exposure. Moreover, in normal patients, peak exposures above accepted daily intake levels for DON were routinely observed without evidence of adverse health effects.

These findings were confirmed in a longerterm trial of measurement of urinary biomarkers for aflatoxins in Brazil.79 16 volunteers, age 14-55 years old collected first morning urine four times every three months from June 2011-March 2012. Aflatoxin M-1 was found in 61% of samples. Residues of aflatoxin metabolites were not identified in any urine sample. GST was not evaluated. There were no differences in aflatoxin measurement over the four seasons of the study.

Sophisticated measurement of urinary biomarkers shows little or no relationship to development of adverse human health effects. In a study from Spain,80 human urine samples were analyzed for 15 mycotoxins and metabolites using a new multi-mycotoxin GCMS/ MS method following salting-out liquid, liquid extraction. 54 urine samples from healthy children and adults in Valencia were analyzed for mycotoxins and were normalized by simultaneous measurement of creatinine. 37 of the 54 samples showed quantifiable values of mycotoxins, finding H-2 toxin, nivalenol and DON. The co-occurrence of these contaminates was seen in 20.4% of samples. 2 of 9 exposed children had levels of DON in urine exceeding international levels without adverse health effects.

Urinary Mycotoxins in Health: Case/Control Studies

In one of the few studies performed looking at occupational exposure of mill workers, an experimental design was adequate to sort out occupational exposure to mycotoxins from diet from three separate grain mills in Germany with matched controls having parallel analysis.81 Mycotoxins tested by urinary measurements were citrinin, DON, ochratoxin A, and zearalenone.

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Immunoaffinity columns and liquid-liquid extraction (ochratoxin) was employed for urine sample clean up prior to liquid chromatography with tandem mass spectrometry (LC-MS/MS) or by high performance liquid chromatography (HPLC). Mycotoxin metabolites analyzed included DON-1, ochratoxin alpha, dihydrocitrinone, alpha- and beta- zearalenone. Urine samples were positive in both groups for citrinin, DON, ochratoxin and zearalenone. DON was found to be the highest concentration in both groups followed by ochratoxin. Mean biomarker levels in urine from mill workers controls were not significantly different, so levels of mycotoxins in urine simply reflected dietary exposure.

Absence of effect of known inhalational exposure on urinary measurements creates a high hurdle for those who espouse significance of urine testing as a reliable marker for illness.

An ongoing problem in measurement of urinary mycotoxins is the possible confounder created by multiple mycotoxins exposure. In a study in South Africa of food and first morning urine, sophisticated LC-MS evaluation82 was able to show correlation of food consumption with presence in morning urine of fumonisins, DON, zearalenone and ochratoxin A. This paper demonstrates the value of multibiomarker measurements in measuring exposures in populations exposed to multiple mycotoxins.

Assessment of simple sample preparation procedures for evaluating mycotoxins in foods and urine were performed with comparison dispersive liquid-liquid microextraction and salting-out-liquid-liquid extraction of analysis of ten fumonisins mycotoxins in metabolites in urine were compared83 (see 84 for tandem study). Under optimal extraction techniques, salting out liquid-liquid extractions showed a better accuracy in precision than dispersive liquid-liquid microextraction. Based on these preliminary results a multi-biomarker method and based on salting out liquid-liquid extraction followed by gas chromatography and tandem mass spectrometry was initiated. The method resulted in low limits of detection and quantitation down to 0.12 and 0.25 micrograms per liter respectively.

A follow-up paper84 from the same group looked at quantitative LC-MS/MS measurement of 11 mycotoxins (aflatoxin, ochratoxin and others in human urine) using dispersive liquid-liquid microextraction methods on ten urine samples from healthy volunteers showed the presence of mycotoxins in low concentration.

This paper from the UK looked at differences in appearance of mycotoxins in urine in healthy adult volunteers and healthy vegetarians.85 Statistically significant differences were seen with 32% of vegetarians exceeding recommended tolerable daily intakes of mycotoxins.

Ongoing enhancements of LC-MS/MS technology are reported in this study from China looking at zearalenone and its metabolites in urine. 301 urine samples were collected from healthy volunteers of all ages in China with 71% of all samples positive for

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zearalenone and metabolites. Adolescents had a higher exposure then children, adults and the elderly.[86]

In another study, one of the few that still uses ELISA kits to assay for aflatoxins87 the study compared urinary aflatoxin measurements in 84 individuals either in a rural or semi-urban community in Nigeria. 99% of urine samples had detectable aflatoxin. Levels were higher in the semi-urban population compared to the rural population. There was no significant difference in mean urinary aflatoxin levels in males/females compared among children, adolescents and adults.

In one of the few studies done comparing mycotoxins found in urine to those in dust, 21 cases who worked in a bread dough factory were compared to 19 individuals who were controls. No reports of illness of either group were found.88

In workers, DON and ochratoxin were the most prevalent biomarkers found in 66% and 90% of participants. In controls DON was found in 58% and ochratoxin in 66%. DON was the mycotoxins measured in highest amounts in settled dust samples. Workers in both groups were exposed to several mycotoxins simultaneously, but there was no difference in urine findings in cases and controls. Exposure in the workplace was not felt to be contributing to adverse health effects. However, the workers did have a higher contact with flour dust which revealed a higher exposure to DON. It becomes problematic to institute risk management when the selected biomarker of mycotoxins in urine has no relationship to illness in studies done with control groups and exposed workers alike.

This is a human biomonitoring study of multiple mycotoxins in urine from Belgium known as the BIOMYCO study.89 This study design assessed mycotoxin exposure in Belgium adults and children using urine for the measure of exposure. Morning urine was gathered according to a standard study protocol involving 155 children and 139 adults. Urine was analyzed for presence of 33 mycotoxins including aflatoxins, trichothecenes and ochratoxin as well as metabolites using LCMS/MS methods. DON and ochratoxin and their metabolites were the most frequently detected. A metabolite of DON, deoxynivalenol-15-glucuronide, was the main DON biomarker and was found in all samples. DON itself was detected in 70% of children and 30% of adults. Ochratoxin was found in 51% of children and 35% of adults. Urinary mycotoxins differed significantly based on age and gender in this study. Biomarker analysis showed a clear exposure of a broad segment of a Belgium population to DON and ochratoxin. The concept of risk assessment arises given that young children may need special attention because there is a relatively higher food intake per kilogram of body weight, so it may be worth examining if the biomarkers present in high amounts are a risk factor. No symptoms were presented that correlate with those amounts.

In an interesting study from the UK, assessment of DON in an elderly cohort90 was taken with 20 patients over the ages of 65 reporting urine findings on two consecutive days. The level of

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quantification was detected in 90% of elderly men and woman on both days. Dietary assessment of DON suggested only 10% of the elderly exceeded the maximum provisional tolerated daily intake for DON. No data on health or illness in these patients was reported.

In a study without human health assessments, forklift drivers at waste management facilities were assessed for occupational exposure of drivers as well as toxicity of dust collected from filters mounted inside forklifts.[91] Mycotoxin analyses were performed by LCMS/ MS methods. Cytotoxicity was assessed using a filter extract which was analyzed using MTT cell culture. Aspergillus species were the predominant organism detected, but no mycotoxins were detected in filter extracts, although those same extracts were either highly toxic or moderately toxic in cell culture. One is left with the question, what in this mixture of dust material besides mycotoxins was creating the cellular injury? Further, if cellular injury were present, did the forklift drivers have evidence of illness? Those questions remain unanswered.

Exposure to mycotoxins is not confined to mammals; in this study of aspergillosis in poultry,92 consideration of the role of gliotoxin was included. Autopsy was performed on 73 birds, all of which presented with an illness consistent of aspergillosis. A culture was done; chloroform extraction of gliotoxin, thin layer chromatography, and histopathology was performed. Aspergillus fumigatus identification was confirmed by PCR. Seven isolates of A. fumigatus were obtained in 6 of them. Gliotoxin-like compounds were detected. Though these numbers are less than 10% of the total avian population, the role of gliotoxin in birds is possibly important.

In a different approach to gliotoxin, the authors93 discuss gliotoxin isolated from Trichoderma species as an antibiotic substance involved in biological control of plant pathogenic fungi. Gliotoxin is felt to be a defense molecule thought to have a role in aspergillosis and is used in Trichoderma-based bio-fungicides. Gliotoxin has medicinal properties as a potential diagnostic marker and is important in biological crop protection. This paper does not assess the claims regarding endogenous production of gliotoxin as an illness-causing agent.

Gliotoxin has a critical role in pathobiology for Aspergillus fumigatus. It modulates the immune response and induces apoptosis in different cell types. This fungal metabolite has been subjected to many investigations with a focus on its biosynthetic pathway.94

In another study of gliotoxins95 that looked at the virulence of Aspergillus fumigatus. Genes in this cluster include a transcription factor and a non-ribosomal peptide synthetase. Two laboratories have reported gliotoxin to be an important virulence determinate, but three other laboratories showed it was not. The disparity was found to be the immunosuppressive regimen used for mice. Gliotoxin was found to be unimportant when immunosuppression with cyclophosphamide and steroids were used. If the

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immunosuppression was accomplished with steroids only, gliotoxin was important using virulence methods. These studies indicate that the mouse model is inadequate to evaluate secondary fungal metabolites in human illness. Questions about gliotoxin determination in urine will remain.

With the focus on the gene clusters of Aspergillus fumigatus for gliotoxin biosynthesis, several important metabolites produced by the gliotoxin biosynthetic pathway were identified.96 These metabolites were influenced by either gliotoxin or specific reactions within the pathway. The activity of gliotoxin against animal cells and fungi was often mediated by interference with redox hemostasis. This is an area where glutamic acid would oppose gliotoxins in animals.

This study[97] alludes to overlap of glutathione transferase (GST) in the gliotoxin biosynthetic pathway. Deletion of one of the genes (Glig) from the cluster (identified as a GST) results in cessation of gliotoxin biosynthesis. Return of Glig restored gliotoxin production in vitro. As we investigate gliotoxin more, its role in induction of GST needs to be clarified.

In an earlier gliotoxin study the gliotoxin biosynthetic pathway was examined using a genomics approach. Differential gene activation in this pathway is co-regulated with timing of expression correlating with production of gliotoxin and culture. This is another in vitro study,98 one without GST, yet suppression of gene activity in the biosynthetic pathway would protect against gliotoxin production.

Gliotoxin exerts a broad spectrum of immunosuppressive effects in vitro and is detectable in serum of patients suffering from invasive aspergillosis.99 No comment is made about the correlation of urinary gliotoxin with serum gliotoxin. This study compared isolation of gliotoxin from 158 aspergillus isolates. There were A. fumigatus; 27 A. terreus, 16 A. niger and A. flavus had 15. Gliotoxin was identified in 98% of A. fumigatus patients with 96% environmental samples. The toxin was also found in 66% of A. niger, 37% of A. terreus and 13% of A. flavus. Culture supernatants of an Aspergillus fumigatus strain lacking gliotoxin showed a significantly lower cytotoxicity on macrophage-like cells than Tcells in vitro. Curiously, lack of gliotoxin production in the other Aspergillus species had no influence on cytotoxic effect of culture supernatant on these immune cells. The study does not speculate on why gliotoxin would show more toxicity from A. fumigatus strains compared to other Aspergillus species.

This study examined ochratoxin and citrinin found in blood in 104 blood samples taken from University students in Bangladesh in 2013 and 2014.100 Ochratoxin was present in all samples. The investigators calculated dietary ochratoxin intake among the students and it was found to be lower than the tolerable weekly intake for ochratoxin. The method used for analysis included LC-MS/MS and HPLC techniques. One can question whether blood versus urine analysis is more accurate. This question is not resolved by the literature.

A study[101] from Germany specifically focused on citrinin looking at urine samples from a

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group of 50 healthy adults (27 females, 23 males). There was a mild increase in urinary citrinin in males compared to females. Finding citrinin and its metabolites in over 80% of all urine samples in healthy patients was instructive.

In another study from Bangladesh,102 ochratoxin and citrinin were evaluated in pregnant woman in Bangladesh. 54 urine samples were collected from residents of rural and suburban areas for analysis using LCMS/ MS methods together with HPLC Ochratoxin was found in 93%, citrinin in 87%. There is suggestion of mild difference between urban participants. Urinary biomarkers for ochratoxin and citrinin did not show significant association with intake of food although there were higher levels of citrinin levels with participants who ate more rice.

In an important study[103] looking at piglets that were feed with Fusarium toxin contaminated maize, assessing presence of mycotoxins found in blood, liquor and urine with LC-MS/MS, a variety of levels of dietary contamination was noted during 29 days of treatment. Concentrations of zearalenone and DON and their metabolites were analyzed. In urine all analytes were detected in significantly higher concentration compared to serum and liquor. The toxin intake for body weight 3-4 hours before slaughter correlated with the sum of DON metabolites in all three specimens as well as with zearalenone. In the first study reviewed for this report, given the high correlation of dietary DON and the measured DON, the exposure can be evaluated. Serum levels of these toxins were indicative of exceeding the guidance value in feed using regression equations. There was significant individual variation among pigs that needs to be considered.

In a variation of animal studies other researchers[104] looked at zearalenone metabolism in dairy cows. The study design included assessment of zearalenone in blood, milk, urine and bile. Interestingly, the bile concentration of measured zearalenone in cases and controls in contaminated feed in cows were not significantly different, suggesting that rumen fermentation mediated alterations in zearalenone and metabolites were associated with alterations of bile formation and bile turnover.

In variation of normal case /control studies in an Italian study,105 a group of 55 celiac patients were compared to a control group of 50 healthy subjects with measurement of DON and zearalenone in 105 urinary samples. Markers were detected in 21 celiac patients and 15 controls corresponding to about 34% of the total participants. There was no statistical difference in mycotoxin exposure in the two groups. These findings do not suggest specific regulation of gluten free products as levels of urinary mycotoxins were no different in celiac patients compared to controls.

As discussed, finding ochratoxin in human blood of healthy patients is not unusual. In a patient study in Tunisia, 106 blood samples from healthy subjects were analyzed using HPLC measurements. Ochratoxin was found routinely. An additional study of ochratoxin in blood did not show any

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correlation with age or gender. The highest ochratoxin plasma levels were found mostly in summer. Ochratoxin levels in populations showed variations from year to year but intra-individual repetition showed no specific trend. No correlation of presence of ochratoxin in human plasma was made with abnormalities in health status.

In a study in the Balkans,107 variations of ochratoxin A in healthy populations were identified. Ochratoxin was measured in 983 samples using HPLC techniques with fluorescent detection. Samples containing ochratoxin above the detection level were found in populations from all Croatian cities at all collecting periods. The highest levels of ochratoxin were in June. While the levels of ochratoxin found in Croatia were lower than other European countries, the study shows that healthy populations of Croatia are exposed to low but seasonally/regionally variable amounts of ochratoxin. 983 samples are the largest study seen in preparation of this review. The conclusions of no evidence of adverse effects from elevated ochratoxin in blood in impressive.

A pediatric population was evaluated in another study108 comparing subjects from Egypt to Guinea, looking for aflatoxin exposure in young children. Using urinary aflatoxin metabolites in parent toxin samples from Guinea (N=50, age 2-4 years) were analyzed with immunoaffinity clean up, followed by HPLC and fluorescent detection. Aflatoxins were less frequently found in Egyptian children (38%), compared to Guinean (86%) children. These specimens of urine were from healthy children, with less exposure apparent in Egypt compared to Guinea. The study concludes that measures to reduce aflatoxin exposure to both regions are important but unfortunately, to support that conclusion, we have no long-term data to look at the risk of association of exposure to illness.

There is another study from Egypt.[109] Looking at aflatoxin, as it constitutes a real human threat, this study enrolled 50 healthy breastfeeding mothers and their infants who were exclusively breast feed for at least 4 months. All had thorough lab evaluations including measurement of aflatoxin by HPLC. Fortyeight % of mothers and their infants had been contaminated with aflatoxin found in mothers' blood, mothers' milk and infants' blood. There was no evidence in this study that the contamination was associated with renal or liver function abnormalities.

Summary of control studies:

21 studies covering 2756 controls from children to adults from North and South America, Europe, Asia and Africa. One study showed positive urine mycotoxins of 60% of 15 patients; one showed 66% of 19 patients with the rest showing 80-100% positive.

In specialized groups, there were 11 studies covering 421 controls. The lowest % positive were 38% in Egyptian children, 48% each for nursing mothers and infants; with the rest being > 75% to 100%.

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Two papers purport to support the urinary mycotoxin hypothesis

A fundamental question for those health care providers who feel that (i) presence of urinary mycotoxins define a new illness and (ii) use of antifungals will treat the illness is, What does exposure to mycotoxins actually mean? If one breathes mycotoxins, there be an immune response adequate to generate the inflammatory responses widely published in CIRS, as described previously in this review, seen in about 25% of patients. All, however, are at risk for a positive urine mycotoxin test. For whom will mycotoxins be ingested, metabolized properly by the human body and excreted harmlessly in the urine? Our multiple cohorts reported herein with good health and positive urine tests showed a marked preponderance of people from around the world that may fall in this category. Or, are mycotoxins generated in unknown ways and unknown locations as defined by an unknown case delineation giving a positive urine test.[8,9]

In nearly all instances, mycotoxins will appear in urine, just as they do in controls. How does one assign weight of causation to a biomarker that does not separate cases from controls? Or are we simply looking at dietary sources of mycotoxins? If so, what is the value of doing urinary testing to diagnose sick people?

As discussed in the section above, a high percentage of control patients show mycotoxins in urine. We saw that not only will trichothecenes, aflatoxin, and ochratoxin routinely appear in urine of controls but their metabolites will as well. These metabolites are readily determined by newer techniques like mass spectrometry, but ELISA testing is fundamentally flawed in that there will be a variety of compounds with similar, but not identical structures, called epitopes, to the quested mycotoxins found in urine. Testing for metabolites of mycotoxins, which one would expect to be mandatory under standard uses of ELISA, would then be skewed as these epitopes would give the false appearance of significance in the urine.

Since metabolites are not reported by two commercial urine mycotoxin test labs in the US, what criteria assist us in ruling out a positive test by presence of a benign metabolite?

We don't know what basis these commercial labs have to define an abnormality as such and not just due to dietary effects that are ubiquitous?

An even greater challenge is what did mycotoxins do on the way through the human body, perhaps through the gastrointestinal tract or the respiratory tract, to get to the urine? Did they set off an immune response, creating a CIRS, or did they metabolize into benign degradation daughters as they were eliminated as waste harmlessly? Mere presence of contaminants in urine is not enough to show causation of illness.

The peer-reviewed literature supporting the use of antifungals and urinary mycotoxins is not non-existent, but certainly is far less robust than

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what we would expect over the past ten years from proponents of the idea trying to establish its validity. The purpose of peerreviewed literature is to present ideas in a rigorous fashion, with rigorous bibliographies that are even and unbiased that will allow the skeptical scientist a mechanism to evaluate the evidence presented. Research papers will usually have an abstract and conclusion; unfortunately, many people will only read those two elements of a published paper. Perhaps a better approach is to look at the methods of a study to see if there is any point in reading the study, because if the methods are illogical or incomplete, there is no point in wasting time reading flawed science, much less investing health care dollars in antifungals or the proponent of their use.

We look for a distinct method section in any published paper. The first paper advocating ELISA methods for mycotoxins in urine was published in 2009 by Dennis Hooper and David Straus. This paper appeared in International Journal of Molecular Sciences with a PubMed citation (8). We see an abstract, introduction, results (methods are not in a specific section), with a conclusion without a stand-alone discussion (there is a section called preparation and evaluation of specimens for mycotoxin detection). This is not a standard design for a research paper. The paper presents urinary findings for ochratoxin, aflatoxin and trichothecenes with no discussion of metabolites. There is no discussion of any of the known congeners for ochratoxins. There is no discussion of epitopes confounding ELISA results found in cases.

The author specifically notes that the experimental design lets them derive qualitative results but not quantitative results. The results claimed that spiked samples created confirmation of the ability of the antibody procedure to detect increasing amounts of toxin; how was this possible if the method wasn't quantitative?

Of vital importance in any kind of test that is being brought to consideration for public use is to compare (i) known cases to (ii) defined controls. Cases are known when they meet a case definition. Hooper and Straus use no case definition. There is no attempt to present a transparent differential diagnosis as no differential diagnosis was presented, with such a process needed to ensure rigor in diagnosis.

No control definition is used. The only control is a negative control group as determined by absent or low mycotoxin levels. One wonders if controls were named simply as a result of a negative test because the world's literature, we looked at had no control groups with less than 38% positive, with most over 80-90%. The paper is silent to this concern.

Since people with exposure to WDB must fulfill four layers of case definition to be called a case, we would expect some sort of algorithm to be presented of how controls were shown not to be exposed. We are told that specimens from patients with no known toxic mold exposures were tested to develop a set of reference data for a control group. There is no table presenting what methods were used to show absence of microbial amplification in buildings for each control or

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presence of amplification for each case.

We are not given ages of the 55 controls, gender of the 55 controls or race of the 55 controls. We are not given any human health data regarding the case samples other than symptoms acknowledged by physicians as being related to mycotoxin mold exposure in and out. This is an egregious error: symptoms are never adequate alone to make a diagnosis without (i) differential diagnosis and (ii) satisfaction of a case definition. It is clear from 25 years of work in the chronic inflammatory response world that the symptoms cited including asthma, memory loss, fatigue, headache, muscle pain or weakness are not specific to exposure to WDB. Indeed, these symptoms are a small portion of the 37 symptoms found in over 30% of CIRS cases as evidenced by published literature beginning in 1997. There is no discussion of validity of symptoms selected by authors in applicability to case definition.

There is no discussion of known biomarkers, well established in peer-reviewed literature, including the US GAO study of 2008; but more importantly published in thousands of cases compared to hundreds of controls beginning in 1998. These publications are not cited in the paper for unknown reasons. Bias as shown by deliberate omission has no basis in science.

Even if the ELISA mycotoxin detection antibodies employed were monoclonal and the author has acknowledged they are not (specific polyclonal antibodies is the term used for aflatoxins; monoclonal for ochratoxin A [congener not specified]; and roridin antibodies for trichothecenes); and even if we can ignore metabolites as possible confounders (we cannot); and even if we felt that the size of the study was adequate to compare to mass spectrometry and liquid chromatography; we are then left to guess as to what the control group actually is. Based on the data presented in the prior section, the likelihood of finding 55 consecutive control patients in Texas without mycotoxins in urine approaches a number of (1/2) to the 55th power. We may conclude that the likelihood of this control group is reliable is not supported.

The next paper by Joseph Brewer and Dennis Hooper, again with a PubMed citation in 2013 (9), reported the detection of mycotoxins in patients with Chronic Fatigue Syndrome. 10 of 104 cases had building sampling (none of results are reported). Note this paper was published after presentation of a case/control study involving hundreds of patients in which biomarkers for CIRS-WDB were presented (22). Use of a previously published diagnostic and treatment protocol was employed with use of vasoactive intestinal polypeptide (VIP) added to enhance patient correction. As seen in the first paper, these published data are not referenced. The author's use a published case definition of Chronic Fatigue Syndrome from Fukuda but given that CFS has no objective biomarkers, none are presented. CIRS-WDB have a host of published biomarkers over the last 25 years: none were included in either study.

Urinary mycotoxin testing was reported to have been used to compare cases to healthy controls,

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previously reported. These controls are now identified for the first time by Dr. Brewer a being 28 males and 27 females, age 18-72 years. These were from diverse geographical areas and resided in various areas of the United States. Control subjects were reportedly asked about complaints and/or symptoms related to mold exposure, but none are reported in a standard data table. It may be assumed the controls had exposure to foods; and airborne mold spores could occur in their daily activity. It is these groups, who nearly always have mycotoxins in urine, as referenced above are found to have trivial levels at best.

There was no delineation of any environmental sampling used to confirm the potential for exposure as required by the US GAO Report of 2008. They do not discuss cases or controls for exposure to water-damaged buildings with musty smells; with visible mold; or with DNA sampling to give accurate delineation of species in genus or fungi present. Without documenting potential, or absence of potential for cases and controls, respectively, no conclusions can be drawn about exposure. Once again, testing for urine mycotoxins (aflatoxins, ochratoxins and macrocyclic trichothecenes) was done, noting that in Hooper's 2009 paper, the antibodies used were against satratoxin and roridin. Extrapolation from these two to DON and others cannot be justified even though there is possible cross reactivity from zearalenone and DON confounding diagnosis for satratoxins.

Testing in cases is done on urine sent in a nonrefrigerated container and analyzed at some time after receipt. No documentation is provided regarding stability of clinical samples by whatever delivery method (not sent on dry ice, not sent on wet ice, not sent overnight) such that we don't know stability of the urine substrate.

The statistics presented ignore metabolites and ignore other types of macrocyclic trichothecenes. There is only documentation of qualitative results in cases published by Hooper in 2009 and yet in 2013 the authors now are claiming ELISA data to be both quantitative and specific. Methods do not disclose the source of conversion of the ELISA from qualitative to quantitative. We find the controls used are the same 55 patients without mycotoxins in urine. The same argument of lack of credibility for this finding applies.

Interestingly in Table 2 the control patients are listed as having no aflatoxin; no ochratoxin and no macrocyclic trichothecenes; indeed, no mycotoxins of any kind were detected and yet in Table 3 now we find that controls do have ochratoxins and macrocyclic trichothecenes These results remain confusing, as they appear to be mutually exclusive.

Brewer states, The environmental histories of these patients for positive exposure to WDB many with visible mold and over 90% of these illnesses tested included residential and workplace. These data are not presented in the paper. Testing was performed only in 10 of 104 patients (data not shown). An analysis of ten cases out of 104 is hardly sufficient to create credibility for history as any experienced mold treating physician knows.

Additionally, this paper quotes the Mitochondrial

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Disease Foundation as a significant reference. Mitochondrial deficiency is listed as the underlying factor causing manifestations including autoimmune disorders, chronic fatigue, neurodegenerative disorders including ALS, MS and Parkinson's disease, depression and psychiatric disorders, together with glycogen disorders among others. There is no basis presented for these claims.

The link to mitochondria, not confirmed for injury to mitoribosomes, claims that mycotoxins cause mitochondrial dysfunction. Whether mitochondria were disrupted in these patients is not clear. One cannot use idle speculation as proof of causation in two patients out of 104.

The conclusions refer to the healthy control population as non-exposed to water-damaged buildings. These are patients with impossible findings in which no mycotoxins are found Regarding the Chronic Fatigue Syndrome patients, a majority had prior exposure to WDB, when in fact, data on only 10 out of 104 were alluded to and less than 5 are reported with unconvincing data. We expect clear and convincing exposure data on all patients, both controls and putative CFS cases. Additional unsupported speculation is that mitochondrial dysfunction is a possible cause for the health problems in these patients and such mitochondrial dysfunction may be triggered and accentuated by exposure to mycotoxins.

Even if we are presented with impeccable lab results from ELISA and thorough use of standard differential diagnosis (we aren't), based on world-wide control data, and a robust literature on CIRS, there is no basis to ascribe any diagnostic significance to urine mycotoxin testing.

Summary:

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REFERENCES:

1.Audenart K, Vanheule A, Hofte M, Haesaert G. Deoxynivalenol: A major player in the multifaceted response of Fusarium to its environment. Toxins 2013, 6: 1-19.

2. Medically sound investigation and remediation of water-damaged buildings in cases of CIRS-WDB. Part 1. Berndtson K, McMahon S, Ackerley M, Rapaport S, Gupta S, Shoemaker R. 10/15. www.survivingmold.com. Accessed 7/23/2019.

3. Ryan J, Shoemaker R. RNA-Seq on patients with chronic inflammatory response syndrome (CIRS) treated with vasoactive intestinal polypeptide (VIP) shows a shift in metabolic state and innate immune functions that coincide with healing. Med Res Arch 2016; 4(7): 1-11.

4. Shoemaker R, Ryan J. Ebook. 3/1/2018. A Gene Primer for Health Care Providers: The Genomics of CIRS and Associated Molecular Pathways: Interpreting the Transcriptomics Results

5. www.mycotoxins.info, accessed 8/21/2019

6. Shoemaker R. Residential and Recreational Acquisition of Possible Estuarine Associated Syndrome: A New approach to Successful Diagnosis and Therapy, Environmental Health Perspectives, Special CDC Pfiesteria Supplement, 2001; 109S5; 791-796.

7. Kawamoto M, Page E. Notes from the field: use of unvalidated urine mycotoxin tests for the clinical diagnosis of illness-US 2014. M MWR 2015; 64(6): 157-158.

8. Hooper D, Bolton V, Guilford F, Straus D. Mycotoxin detection in human samples from patients exposed to environmental molds. Int J Mol Sci. 2009, 10: 1465-1475.

9. Brewer J, Thrasher J, Straus D, Madison R, Hooper D. Detection of mycotoxins in patients with Chronic Fatigue Syndrome. Toxins 2013, 5: 605-617.

10. Jarvi K, Hyvarinen A, Taubel M, Karvonen A, Turunen M, Jalkanen K, Patovirta R, Syrjanen T, Pirinen J, Salonen H, Nevalainen A, Pekkanen J. Microbial growth in building material samples and occupants' health in severely moisture-damaged homes. Indoor Air 2018; 28: 287-297.

11. Arakawa K. Manipulation of metabolic pathways controlled by signaling molecules, inducers of antibiotic production, for genome mining in Streptomyces spp. Antonie Van Leeuwenhoek 2018; 111: 743-751.

12. Wahibah N, Tsutsui T, Tamaoki D, Nishiuchi T. Expression of barley Glutathione S Transferase13 gene reduces accumulation of reactive oxygen species by trichothecenes and paraquat in Arabidopsis plants. Plant Biotech 2018, 35: 71-79.

13. Shoemaker R, Johnson K, Jim L, Berry Y, Dooley M, Ryan J, McMahon S. Diagnostic process for Chronic Inflammatory response Syndrome (CIRS): A consensus statement report of the Consensus Committee of Surviving Mold. Int. Med Rev. 2018, 4(5): 1- 47.

14. Shoemaker R, Rash J, Simon E. Sick Building Syndrome in water-damaged buildings: Generalization of the chronic biotoxin-associated illness paradigm to indoor toxigenic fungi in Johanning E. Editor, Bioaerosols, Fungi. Bacteria, Mycotoxins and Human Health; 2005; pg 66-77.

15 Shoemaker, R IAQA, Las Vegas, Nevada. 10/14/07 Sequential activation of innate immune elements: a health index for people reexposed to water-damaged buildings.

Internal medicine review

Urinary Mycotoxins: A Review of Contaminated Buildings and Food in Search of a Biomarker Separating Sick Patients from Controls

October 2019

16. Shoemaker R. AIHA 6/08. When SAIIE meets ERMI: correlation of indices of human health and building indices. Minneapolis, Minnesota.
17.USGAO 2008 Indoor mold: Better coordination of research on health effects and more consistent guidance would improve Federal efforts.
18. Shoemaker R, House D. SBS and exposure to water damaged buildings: time series study clinical trial and mechanisms; Neurotoxicology and Teratology 2006; 28: 573-588.
19. Shoemaker R, House, D, Ryan J. Neurotoxicology and Teratology doi: 10.1016/j.ntt.2014.06.004. Structural brain abnormalities in patients with inflammatory illness acquired following exposure to waterdamaged buildings: A volumetric MRI study using NeuroQuant.
20. McMahon S, Shoemaker R, Ryan J. Reduction in Forebrain Parenchymal and Cortical Grey Matter Swelling across Treatment Groups in Patients with Inflammatory Illness Acquired Following Exposure to Water-Damaged Buildings. J Neurosci and Clin Res 2016; 1: 1-4
21. Shoemaker R, Katz D, McMahon S, Ryan J. Intranasal VIP safely restores atrophic grey matter nuclei in patients with CIRS. Internal Medicine Review 2017; 3(4): 1-14.
22. Shoemaker R, House D, Ryan J. Vasoactive intestinal polypeptide (VIP) corrects chronic inflammatory response syndrome (CIRS) acquired following exposure to water-damaged buildings. Health 2013; 5(3): 396-401.
23. Ryan J, Wu Q, Shoemaker R. Transcriptomic signatures in whole blood of patients who acquire a chronic inflammatory response syndrome (CIRS) following an exposure to the marine toxin ciguatoxin. BMC Med Genomics 2015; 8, 2015.
24. Cortes-Cantell M. Paul J, Norris E, Bronstein R, Ahn H, Zamolodchikov D, Bhuvanendran S, Fenz K, Strickland S. Fibrinogen and beta-amyloid association alters thrombosis and fibrinolysis: a possible contributing factor to Alzheimer's disease. Neuron 2010; 66: 695-709
25. Zamolodchikov D, Strickland S. A possible new role for AB in vascular and inflammatory dysfunction in Alzheimer's disease. Thromb Res 2016; Suppl 2: S58-61
26. Ahn H, Chen Z, Zamolodchikov D, Norris E, Strickland S. Interactions of B-amyloid peptide with fibrinogen and coagulation factor XII may contribute to Alzheimer's disease. Curr Opin Hematol 2017; 24: 427-431.
27. Khoury A, Atoui A. Ochratoxin A: A general ooverview and actual molecular status. Toxins 2010, 2:461-48. 28 Ponikau J, Sherris D, Weaver A, Kita H. Treatment of chronic rhinosinusitis with intranasal amphotericin B: a randomized, placebo-controlled, double-blind pilot trial. J Allergy Clin Immunol 2005; 115: 125-31.
29. Ebbens F, Georgalas C, Rinia A, van Drunen C, Lund V, Fokkens W. The fungal debate: where do we stand today? Rhinolog 2007; 45: 178-89.
30. Fokkens W, van Drunen C, Georgalas C, Ebbens F. Role of fungi in pathogenesis of chronic rhinosinusitis: the hypothesis rejected. Curr Opin Otolaryngol Head Neck Surg 2012; 20: 19-23.
31. Braun H, Stammberger H, Buzina W, Freudenschuss K, Lackner A, Beham A. Incidence and detection of fungi and eosinophilic granulocytes in chronic rhinosinusitis. Laryngorhinootologie 2003; 82: 330-40.
32. Institute of Food Technologists. 2006 Updates on the science of fungal toxins, 71(5) J Food Sci doi:1

Internal medicine review

Urinary Mycotoxins: A Review of Contaminated Buildings and Food in Search of a Biomarker Separating Sick Patients from Controls

October 2019

0.1111/j.1750-3841.2006.00052. x.).
33. Bosco F, Mollea C. 2012 Mycotoxins in Food, Food Industrial Processes-Methods and Equipment. Dr. Benjamin Valdez (ed). ISBN 978-953-307-905-9).
34. Wallin S, Gambocorta L, Kotova N, Warensjo Lemming E, Nalsen C, Solfrizzo M, Olsen M. Biomonitoring of concurrent mycotoxin exposure among adults in Sweden through urinary multi-biomarker analysis. Food Chem Toxicol 2015; pii: S0278- 6915(15)00188.
35. Shoemaker, RC and Lark, D - 2016, HERTSMI-2 and ERMI: Correlating Human Health Risk with Mold Specific qPCR in Water-Damaged Buildings, #658 in Proceedings of the 14th International Conference on Indoor Air Quality and Climate, International Society for Indoor Air Quality and Climate, Ghent, Belgium.
36. Tu C, Akgul B. Drosophila glutathione Stransferases. Methods Enzymol 2005, 401: 204-26.
37. Gao Y, Cao Y, Tan A, Liao C, Mo Z, Gao F. Glutathione-S-transferase M1 polymorphism and sporadic colorectal risk: An updating metaanalysis and HuGE review of 36 case control studies.
38. Wu M, Xiao H, Ren W, Yin J, Tan B, Liu G, Li L, Nyachoti C, Xiong X, Wu G. Thereutic effects of glutamic acid in piglets challenged with deoxynivalenol. PLOS One 2014; 9: e100591.
39. Duan J, Yin J, Wu M, Liao P, Deng D, Liu G, Wen Q, Wang Y, Qiu W, Liu Y, Wu X, Ren W, Tan B, Chen M, Xiao H, Wu L, Li T, Nyachoti C, Adeloa O, Yin Y. Dietary glutamate supplementation ameliorates mycotoxin-induced abnormalities in the intestinal structure and expression of amino acid transporters in young pigs. PLOS One 2014; 9: e112357.
40. Wu M, Xiao H, Ren W, Yin J, hu J, Duan J, Liu G, Tan B, Xiong X, Oso A, Adeola O, Yao K, Yin Y, Li T. An NMR-based metabolomic approach to investigate the effects of supplementation with glutamic acid in piglets challenged with deoxynivalenol. PLoS One 2014; 9: e113687. doi: 10.371/journal.pone.0133687
41. Kerkadi A, Barriault C, Tuchweber B, Froohlich A, Yousef M. Dietary cholestyramine reduces ochratoxin A-induced nephrotoxicity in the rat by decreasing plasma levels and increasing fecal excretion of the toxin. J Toxicol Environ Health. 1998, 53: 231- 250.
42. Underhill K, Totter B, Thompson B, Prelusky D, Trenholm H. Effectiveness of cholestyramine in detoxification of zearalenone as determine in mice. Bull Environ Contam Toxicol 1995; 54 128-134.
43. Versilovskis A, De Saeger S. Sterigmatocystin: occurrence in foodstuffs and analytical methods—an overview. Mol Nutr Food Res 2010; 1: 136-47.
44. Ates I, Ulker O, Akdemir C, Karakaya A. Correlation of ochratoxin A exposure to urinary levels of 8-hydroxydeoxyguanosine and malondialdehyde in a Turkish population. Bull Environ Toxi 2011; 3: 258-62.
45. Rychlik M. Rapid degradation of the mycotoxin patulin in man quantified by stable isotope dilution assays. Food Addit Contam 2003; 9: 829-37.
46 Rychlik M, Kircher F, Schusdziarra V, Lippl F. Absorption of the mycotoxin patulin from the rat stomach. Food Chem Toxicol 2004; 5: 729-35.
47. Santos R, Vermeulen S, Haritova A, Fink Gremmels J. Isotherm modeling of organic activated bentonite and humic acid polymer used as mycotoxin adsorbents. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2011; 11: 1578-89.

Internal medicine review

Urinary Mycotoxins: A Review of Contaminated Buildings and Food in Search of a Biomarker Separating Sick Patients from Controls

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48. Galvano F, Piva A, Ritieni A, Galvano G. Dietary strategies to counteract the effects of mycotoxins: a review. J Food Prot 2001; 1: 120-31.
49. Schatzmayr G, Zehner F, Taubel M, Schatzmayr D, Klimitsch A, Loibner A, Binder E. Microbiologicals for deactivating mycotoxins. Mol Nutr Food Res 2006; 6: 543- 51.
50. Cserhati M, Kriszt B, Krifaton C, Szoboszlay S, Hahn J, Toth S, Nagy I, Kukolya J. Mycotoxin-degradation profile of Rhodococcus strains. Int J Food Microbiol 2013; 1: 176-85.
51. McCormick S. Microbial detoxification of mycotoxins. J Chem Ecol 2013; 7: 907-18.
52. Molnar O, Schatzmayr G, Fuchs E, Prillinger H. Trichosporon mycotoxinivorans sp. nov, a new yeast species useful in biological detoxification of various mycotoxins. Syst Appl Microbiol 2004; 6: 661-71.
53. Karlovsky P. Biological detoxification of fungal toxins and its use in plant breeding, feed and food production. Nat Toxins 1997; 1: 1-23.
54. Larsson P, Busk L, Tjalve H. Hepatic and extrahepatic bioactivation and GSH conjugation of aflatoxin B1 in sheep. Carcinogenesis 1994; 5: 947 55
55. Hassan Z, Khan M, Saleemi M, Khan A, Javed I, Noreen M. Immunological responses of male white leghorn chicks kept on ochratoxin A (OTA)-contaminated feed. J Immunotoxicol 2012; 1: 56-63.
56. Khatoon A, Zargham K, Khan A, Saleemi M, Javed I. Amelioration of ochratoxin Ainduced immunotoxic effects by silymarin and Vitamin E in white leghorn cockerels. J Immunotox 2013; 1: 25-31.
57. Pestka J. Deoxynivalenol-induced proinflammatory gene expression: mechanism and pathological sequelae. Toxins (Basel) 2010; 6: 1300-17.58. Pestka J, Zhou H, Moon Y, Chung Y. Cellular and molecular mechanisms for immune modulation by deoxynivalenol and other trichothecenes: unraveling a paradox. Toxicol Lett 2004; 1: 61- 73.
59. Pestka J. Mechanisms of deoxynivalenolinduced gene expression and apoptosis. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2008; 9: 1128-40.
60. Zhou H, Jia Q, Pestka J. Ribotoxic stress response to the trichothecenes deoxynivalenol in the macrophage involves the SRC family kinase Hck. Toxicol Sci 2005; 2: 916-26.
61. Lim J, Lee Y, Kim H, Rhyu I, Oh M, Youdim M, Yue Z, Oh Y. Nigericin-induced impairment of autophagic flux in neuronal cells is inhibited by overexpression of Bak. J Biol Chem 2012; 28: 23271-82.
62. Mycotoxin Test Kits | Fast & reliablemycotoxin detection. (2019). Retrieved on September 28, 2019, from https://www.romerlabs.com/en/products/testkits/ mycotoxin-test-kits/.
63. Occurrence, Toxicity, and Analysis of Major Mycotoxins in Food. (2019). Retrieved on September 28, 2019, from https://www.ncbi.nlm.nih.gov/pmc/articles/PM C5486318/.
64. A Brand-New Urine Test for Mycotoxin Exposure The Great Plains .... (2019). Retrieved on September 28, 2019, from https://www.greatplainslaboratory.com/gplblog- source/2017/8/10/a-brand-new-urine-testfor- mycotoxin-exposure.
65. Concentration Levels of Zearalenone and Its Metabolites in Urine .... (2019). Retrieved on September 28, 2019, from https://pubs.acs.org doi/10.1021/jf0113631.
66. Confirmatory analysis method for zeranol, its metabolites and .... (2019). Retrieved on September 28, 2019, from https://library.wur.nl/WebQuery/

Internal medicine review

Urinary Mycotoxins: A Review of Contaminated Buildings and Food in Search of a Biomarker Separating Sick Patients from Controls

October 2019

wurpubs/3168 62.
67. LC. (2019). Retrieved on September 28, 2019, from https://link.springer.com/article/10.1007/s0021 6-013-7011-1.
68. Mycotoxin Analysis in Human Urine by LC. (2019). Retrieved on September 28, 2019, from https://www.ncbi.nlm.nih.gov/pmc/articles/PM C5666377/.
69.Mycotoxin Biomarkers of Exposure: A Comprehensive Review .... (2019). Retrieved on September 28, 2019, from https://onlinelibrary.wiley.com/doi/full/10.111 1/1541-4337.12367.feed-testing/contaminantsand- residues/mycotoxins/.
70.Notes from the Field: Use of Unvalidated Urine Mycotoxin tests .... (2019). Retrieved on September 28, 2019 https://www.cdc.gov/mmwr/preview/mmwrht ml/mm6406a7.htm.
71. Urine as a biomarker of exposure to Fusarium mycotoxins | Food .... (2019). Retrieved on September 28, 2019, from https://www.food.gov.uk/research/researchprojects/ urine-as-a-biomarkers-of-exposure-tofusarium-mycotoxins.
72. Ahn J, et al. (2019). Quantitative determination of mycotoxins in urine by LC. Retrieved on September 28, 2019, from https://www.ncbi.nlm.nih.gov/pubmed/208185 17.
73. Wallin S, et al. (2019). Biomonitoring of concurrent mycotoxin exposure among adults in .... Retrieved on September 28, 2019, from https://www.ncbi.nlm.nih.gov/pubmed/260705 03
74. Capriotti A, Caruso G, Cavaliere C, Foglia P, Samperi R, Lagana A. Multiclass mycotoxin analysis in food, environmental and biological matrices with chromatography/mass spectrometry. Mass Spectrom Rev 2012; 31: 466-503.
75. Li P, Zhang Z, Hu X, Zhang Q. Advanced hyphenated chromatographic-mass spectrometry in mycotoxin determination: current status and prospects. Mass Spectrom Rev 2013; 32: 450-52.
76. Capriotti A, Caruso G, Cavaliere C, Foglia P, Samperi R, Lagana A. Multiclass mycotoxin analysis in food, environmental and biological matrices with chromatography/mass spectrometry. Mass Spectrom Rev 2012; 31: 466-503.
77. Koppen R, Koch M, Siegel D, Merkel S, Maul R, Nehls I. Determination of mycotoxins in foods: current state of analytical methods and limitations. Appl Microbiol Biotechnol 2012; 86: 1595-12.
78. Gerding J, Cramer B, Humpf H. Determination of mycotoxin exposure in Germany using an LC-MS/MS multibiomarker approach. Mol Nutr Food Res 2014; 58: 2358-68.
79. Jager A, Tonin F, Souto P, Privatti R, Oliveira C. Determination of urinary biomarkers for assessment of short-term human exposure to aflatoxins in Sao Paulo, Brazil. Toxins 2014; 6: 1996-2007.
80. Rodriquez-Carrasco Y, Molto J, Manes J, Berrada H. Exposure assessment approach through mycotoxin/creatinine ratio evaluation in urine by GC-MS/MS. Food Chem Toxicol 2014; 72: 69-75.
81. Follmann W, Ali N, Blaszkewicz M. Biomonitoring of Mycotoxins in Urine: Pilot study in Mill Workers. J Toxicol Environ Health 2016; 79: 1015-1025.
82 Shephard G, Burger H, Gambacorta L, Gong Y, Krska R, Rheeder J, Solfrizzo M, Srey C, Sulyok M, Visconti A, Waarth V, van der Westhuizen L. Multiple mycotoxin exposure determined by urinary biomarkers in rural subsistence farmers in the

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former Transkei, South Africa. Food Chem Toxicol 2013; 62: 217-25.
83. Rodriquez-Carrasco Y, Molto J, Manes J, Berrada H. Development of microextraction techniques in combination with GC-MS/MS for the determination of mycotoxins and metabolites in human urine. J Sep Sci 2017; 40: 1572-1582.
84. Escriva L, Manyes L, Font G, Berrada H. Mycotoxin analysis of human urine by LCMS/ MS: A comparative extraction study. Toxins (Basel) 2017; 9:10.3390/toxins9100330
85. Wells L, Hardie L, Williams C, White K, Liu Y, De Santis B, Debegnach F, Moretti G, Greetham S, Brera C, Papageorgiou M, Thatcher N, Rigby A, Atkin S, Sathyapalan T. Deoxynivalenol biomarkers in the urine of UK vegetarians. Toxins (Basel) 2017; 9: doi: 10.3390/toxins9070196.
86. Li C, Deng C, Zhou S, Zhao Y, Wang D, Wang X, Gong Y, Wu Y. High-throughput and sensitive determination of urinary zearalenone and metabolites by UPLC-MS/MS and its application to a human exposure study. Anal Bioanal Chem 2018; 410: 5301-5312.
87. Ezekiel C, Oyeyemi O, Ayeni K, Oyeyemi I, Nabofa W, Nworzichi C, Dada A. Urinary aflatoxin exposure monitoring in rural and semi-urban populations in Ogun state, Nigeria. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2018; 35: 1565-1572.
88. Viegas S, Assuncao R, Nunes C, Osteresch B, Twaruzek M, Kosicki R, Grajewski J, Martins C, Alvito P, Almeida A, Viegas C. Exposure assessment to mycotoxins in a Portuguese fresh bread dough company by using a multi-biomarker approach. Toxins (Basel) 2018; 10: pii: E342. Doi: 10.3390/toxins10090342
89. Viegas S, Assuncao R, Nunes C, Osteresch B, Twaruzek M, Kosicki R, Grajewski J, Martins C, Alvito P, Almeida A, Viegas C. Exposure assessment to mycotoxins in a Portuguese fresh bread dough company by using a multi-biomarker approach. Toxins (Basel) 2018; 10: pii: E342. Doi: 10.3390/toxins10090342
90. Papageorgiou M, Wells L, Williams C, White KLM, De Santis B, Liu Y, Debegnach F, Milano B, Moretti G, Greetham S, Brera C, Atkin S, Hardie L, Sathyapalan T. Occurrence of deoxynivalenol in an elderly cohort in the UK: a biomonitoring approach.
91. Viegas C, Faria T, de Oliveira A, Caetano L, Carolino E, Quintal-Gomes A, Twaruzek M, Kosicki R, Soszczynska E, Viegas S. A new approach to assess occupational exposure to airborne fungal contamination and mycotoxins of forklift drivers in waste sorting facilities. Mycotoxin Res 2017; 33: 285-295.
92. de Oca V, Valdes S, Segundo C. Gomez G, Ramirez J, Cervantes R. Aspergillosis, a natural infection in poultry: Mycological and molecular characterization and determination of gliotoxin in Aspergillus fumigatus isolates. Avian Dis 2017; 61: 77-82
93. Scharf D, Brakhage A, Mukherjee P. Gliotoxin—bane or boon? Environ Microbiol 2016; 18: 1096-1099.
94. Scharf D, Heinekamp T, Remme N, Hortschansky P, Brakhage A, Hertweck C. Biosynthesis and function of gliotoxin in Aspergillus fumigatus. Appl Microbiol Biotechnol 2012; 93: 467-72.
95. Kwon-Chung K, Sugui J. What do we know about the role of gliotoxin in the pathobiology of Aspergillus fumigatus? Med Mycol 2009; 47 Suppl 1: S97-103.
96. Dolan S, O'Keeffe G, Jones G, Doyle S. Resistance

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is not futile: gliotoxin biosynthesis, functionality and utility. Trends Microbiol 2015; 23: 419-28.
97. Davis C, Carberry S, Schrettl M, Singh I, Stephens J, Barry S, Kavanagh K, challis G, Brougham D, Doyle S. The role of glutathione S-transferase GliG in gliotoxin biosynthesis in Aspergillus fumigatus. Chem Biol 2011; 18: 542-52.
98. Gardiner D, Howlett B. Bioinformatic and expression analysis of the putative gliotoxin biosynthetic gene cluster of Aspergillus fumigatus. FEMS Microbiol Lett 2015; 248: 241-8.
99. Kupfahl C, Michalka A, Lass-Florl C, Fischer G, Haase G, Ruppert T, Geginat G, Hof H. Gliotoxin production by clinical and environmental Aspergillus fumigatus strains. Int J Med Microbiol 2008; 298: 319-27.
100.. Ali N, Hossain K, Degen G. Blood biomarkers of citrinin and ochratoxin A exposure in young adults in Bangladesh. Mycotoxin Res 2018; 34: 59-67.
101. Ali N, Blaszkewicz M, Degen G. Occurrence of the mycotoxin citrinin and its metabolite dihydrocitrinone in urines of German adults. Arch Toxicol 2015; 89: 573-8. 102. Ali N, Blaszkewicz M, Manirujjaman M, Degen G. Biomonitoring of concurrent exposure to ochratoxin A and citrinin in pregnant women in Bangladesh. Mycotoxin Res 2016; 32: 163-72.
103. Brezina U, Rempe I, Kersten S, Valenta H, Humpf H, Danicke S. Diagnosis of intoxications of piglets fed with Fusarium toxin-contaminated maize by the analysis of mycotoxin residues in serum, liquor and urine with LC-MS/MS. Arch Anim Nutr 2014; 68: 425-47.
104. Danicke S, Keese C, Meyer U, Starke A,Kinoshita A, Rehage J. Zearalenone (ZEN) metabolism and residue concentrations in physiological specimens of dairy cows exposed long-term to ZEN-contaminated diets differing in concentrate feed proportions. Arch Anim Nutr 2014; 68: 492-506.
105. Cirlini M, Mazzeo T, Roncoroni L, Lombardo V, Elli L, Bardella M, Agostoni C. Doneda L, Brighenti F, Dall'Asta C, Pellegrini N. Are Treated Celiac patients at risk for mycotoxins? An Italian case-study. Toxins (Basel) 2016; 9: pii: E11. doi: 10.3390/toxins9010011.
106. Karima H, Ridha G, Zied A, Chekib M, Salem M, Abderrazek H. Estimation of Ochratoxin A in human blood of healthy Tunisian population. Exp Toxicol Pathol 2010; 62: 539-42.
107. Peraica M, Domijan A, Matasin M, Lucic A, Radic B, Delas F, Horvat M, Bosanac I, Balija M, Grgicevic D. Variations of ochratoxin A concentration in the blood of healthy populations in some Croatian cities. Arch Toxicol 2001; 75: 140-4.
108. Polychronaki N, Wild C, Mykkanen H, Amra H, Abdel-Wahhab M, Sylla A, Diallo M, El-Nezami H, Turner P. Urinary biomarkers of aflatoxin exposure in young children from Egypt and Guinea. Food Chem Toxicol 2008; 46: 519-26.
109. Hassan A, Sheashaa H, Abdel M, Ibrahim A, Gaber O. Does aflatoxin as an environment mycotoxin adversely affect the renal and hepatic functions of Egyptian lactating mothers and their infants? A preliminary report. Int Urol Nephrol 2006; 38: 339-42.
110. B. Warth, M. Sulyok, et al: Development and validation of a rapid multi-biomarker liquid chromatography/tandem mass spectrometry method to assess human exposure to mycotoxins. Mass Spectrom. 2012, 26, 1533–1540