

HERTSMI-2 and ERMI: Correlating Human Health Risk with Mold Specific qPCR in Water-Damaged Buildings

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SUMMARY

In this large study of fungal DNA testing by MSQPCR, we present the findings that support use of low cost HERTSMI-2 testing to inform objectively interested parties

- **If WDB conditions exist; and**
- **Where the problems are likely to be found; as well as**
- **Whether the remediated building is likely to be safe for re-occupancy by previously affected patients with CIRS-WDB who meet the GAO case definition.**

PRACTICAL IMPLICATIONS

While high scores of both ERMI and HERTSMI-2 accurately predicted markedly increased risk of recrudescence, only low HERTSMI-2 predicted safety from re-exposure for patients who had prior CIRS-WDB. Use of HERTSMI-2 is inexpensive, reproducibly reliable and predictive of mold associated re-exposure from water damaged buildings (WDB), especially for sub-optimally remediated buildings.

KEYWORDS

WDB Water Damaged Buildings

CIRS-WDB Chronic inflammatory response syndrome acquired following exposure to the interior environment of water-damaged buildings (WDB)

ERMI Environmental Relative Moldiness Index

HERTSMI-2 Health Effects Roster of Type Specific (Formers) of Mycotoxins and Inflammagens, Version 2

MSQPCR Mold Specific Quantitative Polymerase Chain Reaction

1. INTRODUCTION

In the absence of published governmental guidelines setting criteria for safety in buildings with a history of water intrusion and microbial growth (WDB), clinicians caring for patients sickened by chronic inflammatory response syndrome (CIRS-WDB) have used a variety of building parameters to predict safety of re-exposure, without acceptable predictive success.

Previously, no single building index has consistently shown reliability to predict absence of recrudescence with re-exposure. Therefore, patients with a history of CIRS-WDB have often needlessly experienced recurrence of symptoms following re-exposure to WDB, even with exposures as short as 30 minutes.

Previous studies have shown that the Environmental Relative Moldiness Index (ERMI) has use in predicting re-acquisition of abnormal inflammatory markers of CIRS-WDB with re-exposure to buildings with an ERMI equal to or greater than 2.01 but no assessment of ERMI to predict absence of relapse with re-exposure has been forthcoming. Moreover, ERMI has been criticized as having methodological and mycological problems. In an attempt to

improve predictive value of fungal MSQPCR data as the basis for an accurate building safety index, a derivative of ERMI, called HERTSMI-2, was developed.

HERTSMI-2 uses a weighted scale applied to the concentration in Spore Equivalents/mg of each target mold's DNA, detected by MSQPCR, present in collected dust for just five species of fungi. This index was developed following statistical assessment of 1010 ERMI results from the homes of treated patients (Shoemaker, 2011). Prospectively, HERTSMI-2 was compared to ERMI in the assessment of 807 consecutive patients for whom health effects of re-exposure to buildings were known. These data showing the relevant predictive value of each index is now presented. 618 buildings had ERMI done, from which HERTSMI-2 is calculated; these data were compared to those from buildings where HERTSMI-2 alone was performed (N=189).

Published data has confirmed that the diagnosis, through blood tests of patients sickened following exposure to the interior environment of a water-damaged building (WDB), is readily achievable (Shoemaker, 2013). Use of a standardized treatment protocol, confirmed by double blinded, placebo controlled clinical trial (Shoemaker, 2006), has not only provided resolution of the chronic inflammatory response syndrome (CIRS-WDB) but also provided an opportunity to employ re-exposure trials to determine if the gold standard of remediation, confirmation of absence of recrudescence of illness with re-exposure following thorough remediation, has been met. With increasing use of MSQPCR testing by physicians treating CIRS-WDB patients, we sought to determine a method of measuring successful remediation based on maintenance of resolution of symptoms and laboratory measures in previously affected, but treated CIRS-WDB patients, after re-entry. This method focuses on patient health parameters as a measure of safety of occupation of a building.

The search for a new, objective method to assess safety of remediation for previously affected patients was spurred by failure to see objective, patient-driven data that showed benefit from measures derived from air sampling. Problems with air sampling with spore traps have been reported (GAO, 2008 & WHO, 2009). Low sample volumes and the absence of the ability to microscopically determine the species of spores collected by spore trapping have been amongst the reported causes. While spores of *Chaetomium* and *Stachybotrys* are obvious to skilled microscopists reviewing spore trap material, separation of *Penicillium* from *Aspergillus* is not possible, nor is there a routine mechanism to similarly identify *Wallemia sebi* in spore trapping by microscopy. However, methods to overcome these issues have been evolving.

BACKGROUND to PCR

PCR was invented in 1985 by Kary B. Mullis; use of PCR has become widely applied in almost every field of biological endeavour, truly revolutionizing molecular biology. Its specificity, efficiency and fidelity have turned it into a key technology that has made molecular assays globally accessible. It underpins most of the spectacular advances that are now commonplace in every biological disciplines, ranging from microbial detection and microbiological quality assurance, through the detection of genetically-manipulated organisms in crops and foods, to molecular and veterinary medicine.

Conventional PCR is a qualitative assay, giving a binary presence/absence result, while quantitative, real-time PCR (qPCR or MSQPCR) is a powerful technique that enables both qualitative, as well as quantitative, measurements of specific sequences in a nucleic acid sample. Since various experimental parameters can have a significant impact on the quality of results (in some cases erroneous), it is particularly important to employ standardized best

practices. Those include the use of rigorous controls, validation and non-subjective data interpretation.

ERMI INTERPRETATION OF MSQPCR DATA

To interpret the data offered by MSQPCR in a WDB context, the Environmental Relative Moldiness Index (ERMI) has been developed and validated as a means of interpreting results from MSQPCR of house dust. ERMI was developed by the *U.S. Environmental Protection Agency* (Haugland & Vesper, 2002; Vesper, 2007). The method employs Mold Specific Quantitative Polymerase Chain Reaction (MSQPCR) methods to detect and quantify species of fungi found in WDB compared to those found in buildings without a history of water intrusion.

The MSQPCR method follows defined steps. During the annealing step, the primers and probe hybridize to the complementary DNA strand in a sequence-dependent manner. Because the probe is intact, the fluorescent reporter and quencher are in close proximity and the quencher absorbs fluorescence emitted. In the extension step, the polymerase begins DNA synthesis, extending from the 3' ends of the primers. When the polymerase reaches the probe, the exonuclease activity of the polymerase cleaves the hybridized probe. As a result of cleavage, the fluorescent dye is separated from the quencher and the quencher no longer absorbs the fluorescence emitted by the dye. This fluorescence is detected by the real-time PCR instrument. Meanwhile, the polymerase continues extension of the primers to finish synthesis of the DNA strand.

CLINICAL APPLICATION OF ERMI & EMERGENCE OF HERTSMI-2

Use of ERMI was clearly helpful clinically as elevated ERMI scores indicated absence of safety of homes for those patients with CIRS-WDB. For ERMI scores less than 2.1, the value of ERMI was less likely to correlate with safety.

In order to address this, HERTSMI-2 was initially presented (Shoemaker, 2011), based on a review of over 1000 ERMI test results. Patients were stratified by total ERMI score finding that scores over 2.0 were associated with illness for those with levels of melanocyte stimulating hormone (MSH) < 35 pg/ml or those with HLA DR from one of six genetically predisposing haplotypes (Shoemaker, 2005).

In an effort to find significance of differences between high versus low ERMI, ratios of Spore Equivalents/mg dust derived by MSQPCR were compared for each species listed in Group I of ERMI. The goal was to isolate the minimum number of filamentous fungal species routinely associated with damp buildings that made susceptible patients ill with re-exposure.

Any ratio less than 10/1 for a given species was not considered to be strong enough to be an indicator of worsening building health. Nine species with ratios of greater than 10 were identified. Of these, the five with the highest ratios were (in order) *Wallemia sebi*; *Aspergillus versicolor*; *Aspergillus penicillioides*; *Stachybotrys chartarum* and *Chaetomium globosum*.

Of interest, these organisms stratify water activity (A_w), with A_w ranging from near xerophilic (*Wallemia*) to approaching saturated (*Stachybotrys* and *Chaetomium*).

HERTSMI-2 IS MORE PRACTICAL

In theory, HERTSMI-2 values could provide an inexpensive, objective measure of organisms routinely found in WDB, known to be associated with adverse human health effects. These data could also serve as indicators for remediators as to what conditions and locations were present that were consistent with the A_w of the identified organism. If no conditions were

identified that suggested the presence of excessive levels of *Wallemia*, for example, then additional searching for such conditions must be enjoined.

A further concern is that residences were solely included in the development and validation of ERMI, while other buildings, such as workplaces and schools are no less affected by water intrusion. These have been rarely studied, so there is no data published on any patients re-exposed to workplaces and schools that would contradict the hypothesis presented in early CIRS studies (Shoemaker, 2005) that “wet buildings are wet buildings”.

HERTSMI-2 IN CONTEXT

In “Consensus of Medical Professionals’ Panel” (2015), accessed on www.survivingmold.com, Table 2 shows a fully referenced list of the toxins, inflammagens and microbial products found in WDB. Many of those bio-markers are analyzable but have not been supported by published validation for the purposes of developing a building index. In addition, they are expensive and not widely in demand.

HERTSMI-2 –PROSPECTIVE DATA COLLECTION

Alternatively, here we present a study showing results of fungal DNA testing by MSQPCR and our findings that support use of readily available and low cost HERTSMI-2 testing to inform objectively all interested parties (i) if WDB conditions exist; and (ii) where the problems are likely to be found; as well as (iii) whether the remediated building is likely to be safe for re-occupancy by patients who meet the case definition (GAO, 2008).

2. MATERIALS/METHODS

A total of 807 consecutive MSQPCR studies were collated from charts of patients evaluated in one clinic specializing in diagnosis and treatment of patients affected by WDB. Written informed consent was provided by all participants. Dust samples were collected according to established criteria (Haugland & Vesper, 2002). The MSQPCR analyses were performed by Mycometrics, Inc, Monmouth Junction, NJ. ERMI scoring was supplied by Mycometrics. HERTSMI-2 scoring performed using 2011 algorithm (www.survivingmold.com; HERTSMI-2 scoring table). Patients were admitted to the study only when diagnosed as CIRS-WDB, having met the case criteria established by the US GAO.

The criteria include:

- (1) confirmation of exposure;
- (2) presence of symptoms seen in patients in peer reviewed papers;
- (3) presence of relevant laboratory abnormalities seen in patients, as published in peer reviewed papers; and
- (4) response to treatment, previously present before treatment with the standard protocol, but absent after treatment.

The study was double-blinded; neither patients nor investigators were aware of MSQPCR scores before building re-entry.

Patients were treated with initial steps of a standard protocol (Shoemaker, 2013) including removal from exposure; use of anion binding resins for at least one month and treatment of commensal, biofilm-forming, multiply antibiotic resistant coagulase negative staphylococci (MARCoNS) if found in deep aerobic nasal space. Patients were considered to have relapsed with re-exposure within four hours if they noted reappearance of at least four symptoms.

3. RESULTS

Table 1 provides data representing 618 ERMI scores were identified. No ERMI result was listed for 186 qPCR results as these were resulted using HERTSMI-2 only. Comparison of data obtained with HERTSMI-2 calculated from ERMI is compared to data from HERTSMI-2 without performance of ERMI (Table 2).

Table 1 Grouped ERMI Scores, correlated with Relapse & Building Type

ERMI	N=	Relapse	No Relapse	Relapse %	Building Type 1 N=	Building Type 2 N=	Building Type 3 N=
-8.39-0	49	5	44	10.2	44	2	3
0.01-2.00	40	7	33	17.5	33	3	4
2.01-5.00	87	21	66	24.1	82	3	2
5.01-8.00	89	35	54	39.3	75	3	11
8.01-11.00	77	52	25	67.5	67	4	6
11.01-14.00	82	74	8	90.2	68	8	6
14.01-17.00	65	59	6	92.3	54	5	6
> 17.01	129	127	2	98.4	118	3	8
	618	380	238		541	31	46

Of the ERMI patients < 2.01, 77 did not relapse and 12 did. For ERMI > 2.01, 368 relapsed and 161 did not.

Table 2 Grouped HERTSMI Scores, correlated with Relapse

HERTSMI-2	From ERMI N=	Relapse N=	% Relapse	From HERTSMI-2 only N=	Relapse N=	% Relapse
0-10	181	5	2.7	60	1	1.7
11-15	98	47	48	28	12	42
>15	339	339	100	101	99	99
TOTAL	618	391		189	112	
Total relapse = 503. No relapse = 304						

807 HERTSMI-2 scores are presented, with 618 in ERMI and 189 without ERMI. Low scores (≤ 10) correlated with absence of relapse in 235; relapse was seen in 6 (Table 2). For indeterminate HERTSMI-2 scores (11-15), 59 relapsed and 67 did not. For high HERTSMI-2 (>15), all but 2 of 438 patients relapsed. There were no differences between HERTSMI-2 calculated with or without performance of ERMI. There were no differences between building types 1, 2, 3 (data not shown but similar to Table 1).

The distribution of building types strongly favored residences, with 705 buildings being residences (Building Type 1). 52 workplaces (Building Type 2) and 40 schools (Building Type 3) are also represented in the data set. Relapse and absence of relapse was not significantly different for any building type ($p < 0.01$). Mean ERMI and HERTSMI-2 scores were not significantly different for any building type ($p < 0.01$) (see Table 3).

Table 3 Mean ERMI Scores, correlated with Building Type

Building Type	1	2	3
Mean ERMI	7.3	8.4	10.2
Mean HERTSMI-2	17.6	15.5	17.8

4. DISCUSSION

Indoor Air Quality professionals and health care providers alike continue to search for definitive criteria that can identify a building as safe for human use, or not. Understanding that only 24% of the population at large carries the HLA DR haplotypes associated with increased relative risk for illness following exposure to the interior of WDB (Shoemaker, 2005), it is difficult to apply a specific health effects criterion to all individuals. Further, we cannot use any one single element of those found inside WDB as specifically causing human illness, given the multiple possible sources of antigens, toxins and inflammagens that can each lead to CIRS-WDB. Against the seemingly impossible task required to assign criteria to patients and also to buildings, each for their own reasons, we studied previously affected patients who voluntarily re-entered buildings during medical supervision.

Both ERMI and HERTSMI-2 do not provide information regarding bacteria, actinomycetes and microbial volatile organic compounds (mVOCs). ERMI has a high percentage of errors when predicting absence of relapse (12/89 incorrect) and prediction of relapse (161/529 were incorrect). Total errors were 173/618 (28%). For HERTSMI-2 below 10, there were far less errors when predicting absence of relapse found (6/241); and errors predicting definite relapse at 2/438. However, HERTSMI-2 scores between 11 and 15 were shown to be unreliable for prediction, as such scores showed 59 relapsers and 67 non-relapsers. Such values deserve the appellation of indeterminate.

5. CONCLUSIONS

The evidence presented confirms that data from MSQPCR testing can alert patients with CIRS-WDB and their health care providers to possible problems with re-entry to previously affected WDB. Use of HERTSMI-2 is confirmed to show predictive accuracy of over 97% for patients with low or high scores. Indeterminate values demand additional building evaluation and remediation before permitting re-entry of patients with previously confirmed CIRS-WDB. Given the low cost (~US \$150) and rapid turnaround provided by mycology labs that satisfy all MSQPCR testing requirements, HERTSMI-2 testing can avoid dangerous exacerbation of health effects for buildings with high HERTSMI-2 scores and provide reasonable expectations for safety with cautious re-entry when the HERTSMI-2 scores are low (<10).

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