Indoor exposure to *Streptomyces albus* and *Aspergillus versicolor* elevates the levels of spore-specific IgG, IgG1 and IgG3 serum antibodies in building users — A new ELISA-based assay for exposure assessment

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1. Highlights

- The indoor microbe exposure elevated the building user's serum spore-specific IgG
- A new ELISA system with microbe spore antigens was utilized for the analysis
- *S. albus*- and *A. versicolor* spores were selected for the test antigens
- The individuals from the damaged buildings had increased serum IgG1 and IgG3 levels

2. Abstract

Moisture-indicative microbes in buildings are associated with a variety of symptoms, ranging from mild irritation to severe clinical illnesses. These symptoms are caused principally by dried, dormant and dead microbe material like spores, mycelium and microbe metabolites, leading to the activation of the immune system and formation of the antigen-specific immunoglobulins. This activation presumably takes place through the respiratory track and is a normal immune reaction against pathogenic invaders. During continuous exposure, a prolonged state of inflammation will follow, and this forms a considerable health risk for a building's occupant. A new ELISA system utilizing spores from two species *Streptomyces albus* and *Aspergillus versicolor* as an antigen was developed to reveal the related immunological processes. In 159 persons, microbial exposure was observed to increase the levels of spore-specific IgG, IgG1 and IgG3 serum antibody levels of individuals residing in microbe-dense buildings compared with the control reference buildings. No differences were detected in the levels of *S. albus*- and *A. versicolor*-specific serum IgA or IgM levels.

3. Keywords

Indoor mould and bacteria,

Spore

mycelium

Exposure

Immunoglobulin

4. Abbreviations

ELISA: enzyme linked immunosorbent assay

APC: antigen-presenting cell

Ig: immunoglobulin

Th: T helper lymphocyte

HRP: horseradish peroxidase

OD: optical density

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5.1 Author Conribution:

Janne Atosuo, the writer of this article has been planning and organizing the study and participated to the lab work.

Outi Karhuvaara and Eetu Suominen are responsible for the main laboratory work.

Liisa Vilén has collected the blood samples and participated to the data analysis.

Professor Jari Nuutila has provided the immunological expertise.

Professor, MD, Tuula Putus is the group leader, the expert in environmental and occupational medicine and the main planner of this study.

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7. Conflict of Interest

The authors have no conflicts of interest to declare.

8. Ethics

The study plan was approved by the ethical committee of the Turku University Central Hospital (Dnro: 59/1801/2019)
There were no significant ethical issues with this study. Participation was voluntary, all participants were adults and voluntarily made their information available for research purposes.

9. Introduction

Moisture-indicative indoor microbes, moulds and bacteria are known to be harmful to building users[1, 2]. One of the main routes of exposure is via the indoor air, where the dry

dormant and dead material, like the spores and fragments of mycelium can be found[2, 3] . Exposure is most likely to occur through the respiratory track caused by these dried microbial particles and fragments. Exposure via indoor air is relevant, because 90% of a building user's time is spent indoors[4]. Moisture damage related indoor microbes have an adverse health effects, such as the irritation of airways, skin and eyes, general symptoms, and sometimes they cause fungal sinusitis and invasive infections. People with long-term exposure to moisture damage and indicator microbes have a higher risk for asthma, allergic alveolitis and organic dust toxic syndrome[5-13] . Infections caused by the yeast and filamentous fungi are more prevalent among susceptible population groups. Immunocompromised patients and those under the corticosteroid treatment are at a higher risk for developing deep fungal infections[14] . Nevertheless, exposure to fungi and bacteria may sensitize individuals, cause mucosal irritation and inhibit cilia function in airways[15] and altogether may allow for a greater susceptibility to common respiratory infections, such as common colds, otitis and sinusitis[16-20] .

Relatively little is known about the activation of immune defence by exposure to microbe-damaged buildings, as the damage is usually well-advanced when detected, and the original causative factor may thus remain undetected. It is also common that the primary exposure may have occurred much earlier or even in a different building and sometimes outdoors. Moreover, there are great interindividual differences in responses, and there are no distinguishing patterns about how the exposure causes the immunological activation to proceed.

Our immune system continuously encounters invading microbes and other antigens, which are first recognized and swiftly destroyed by the innate immunity, and only a very small fraction of these encountering actually activate the adaptive arm of the immune system[21-23] . The antigen-specific immunoglobulins (Ig) secreting B cells (plasma cells) are formed in the secondary lymphatic tissue in a course of 1-2 weeks after the primary antigen encountering[21] .

Igs or antibodies are bi-functional molecules, binding to antigens with high specificity and initiating other biologic functions[21, 22] . Ig binding directly neutralizes toxins and they are (IgG) the activators of the serum classical complement system pathway. One of the main functions of Igs is the opsonisation, which is the promotion of the target (e.g., particle, spore, microbe cell) to the phagocyte, a necessary preliminary step of ingestion, internalization and the destruction of the target[24] .

The outcome of the Ig responses is regulated by the ambiguous interactions between the innate and acquired immune systems including the antigen presenting cells (APC) and consequently the cytokines secreted by Type 1 T helper (Th1) or the Type 2 T helper (Th2) populations. The selection between the Th1 or Th2 response is based on the characteristics of the antigen, APC and the interindividual differences[25, 26] .

The development of Th2-triggered and IgE-derived allergic hypersensitivity reactions and asthma[27] linked to the indoor microbes is relatively rare, and the exposure to a microbe-damaged environment increases the risk of non-allergic (i.e., intrinsic) asthma instead of systemic IgE-positive hypersensitivity[28] . In Finnish materials, approximately

20-24 % of the exposed individuals develop IgE-antibodies and also equal share of asthmatics are IgE-mediated [6]. The immunopathology of these two types are similar but the intrinsic reactions are localized in the secondary immune tissues of the lungs and are not visible systemically in normal allergy tests [29-31].

The Th1-regulated proinflammatory action is a key factor in the adaptive humoral and cellular response against the invading microbes. This response is formed by the production of firstly IgM-secreting cells at the initial stage and after the class switch, a more specific IgA or IgG production targeting the intruder for quick removal from the tissue via phagocytosis by polymorphonuclear leukocytes[21] . IgG is the main effector in serum and the IgA response is directed toward the mucosa. A variety of Ig subclasses (i.e., IgA1, IgA2, IgG1, IgG2, IgG3, IgG4) focuses the response in a more accurate and specific direction.

In this study, we assessed the Ig production caused by the dry dormant and dead microbial material in microbe-damaged buildings by using our original, spore-specific enzyme linked immunosorbent assay (ELISA). Our aim was to show that this activation of the immune system can be assessed by using spores as an antigen and moreover, using spores simultaneously from two different species as a combined antigen. Two species were selected based on their prevalence in microbe damages in Finland, one bacteria *Streptomyces albus* [32] and mold *Aspergillus versicolor* [33-35] . Both are so-called indicator species indicating a severity of the damage[36] . The detection and the proof of the exposure is an important step from preventing building users from further exposure, since the

elevated Ig levels are a significant signal for the necessity of the environmental improvement.

10. Materials and Methods

10.1 Buildings

Damages in the building were evaluated by microbe cultivations, and *S. albus* [32] and *A. versicolor* [33-35] were found in all three damaged buildings. More inclusive data from target buildings is shown in Table 1. No microbe damage were observed in four non-damaged buildings (Table 1). Main research material consisted of 159 individual serum samples both from damaged and reference buildings. These are presented in tables 1 and 2.

10.2 Reference samples for test system evaluation

Reference samples were four serum samples from healthy volunteers, who were not included in the study material. Two adult females (aged 28 and 52 years old) and 2 adult males (aged 34 and 48 years old) volunteered.

10.3 Serum sampling and storage

Blood samples were collected from the volunteers in 8-ml Vacuette serum tubes (with gel) (Greiner Bio-one, Kremsmünster, Austria). Serum was prepared by centrifuging at 1800 rpm for 10 min and then distributed into 2-ml Eppendorf tubes and stored at -80°C. Samples were collected while the volunteers were working or living in the buildings.

10.4 Spore-antigen preparation

S. albus spores were cultured and harvested from the mannitol salt agar (MS-agar)[37] , and *A. versicolor* SL/3 spores were cultured and harvested from malt agar[38] and both suspensions were diluted with distilled water[37] . These suspensions were then sonicated in order to dismantle the aggregates according to the standard protocol by Kieser et al.[37]. Spores were isolated from the other possible microbe derived material by filtration through autoclaved cotton wool and re-suspended in 20% glycerol[37] for the freezer stock. The filtration was operated with the cotton wool inside the 20 ml syringe (syringe and wool autoclaveted) by pressing the sonicated suspension trough the wool. Spores passes the cotton wool to the collection tube while other material and debris is immobilized in the wool. The spore suspension was microscoped and the purity, concerning the unwanted debris, was observed. The counting of the spore suspension was not feasible because of their small size and huge number and dry weight determination was used to quantify the spore "mass" instead. This weight was determined by pipetting 1 ml of the spore freezer-stock suspension on the weighted petri dish, which was re-weighted after the evaporation. The spore viability was established by plating the spores before and after the drying and the freezing. No significant loss of viability was observed during these processes (data not shown).

The freezer stock concentration of *S. albus* was 55 g/l and *A. versicolor* 120 g/l (dry weight of the spore suspension) and 100µl of 1/100 dilution in phosphate-buffered saline (PBS; pH 7.4) was used in microtiter well coating. The combination antigen solution was prepared by mixing 50/50 of 1/100 diluted *S. albus* and *A. versicolor* spores.

The *S. albus* wild type strain was kindly donated by the Antibiotic Biosynthetic Engineering Group in the Department of Biochemistry of the

University of Turku, Finland and the *A. versicolor* SL/3 strain by the Department of Aerobiology of the University of Turku, Finland.

10.5 Determination of the spore-specific antibodies by ELISA

Serum antibody levels against spores from species *S. albus*, *A. versicolor* and the combination antigen were determined. Microtitre ELISA plates (Nunc-Immuno plate with Maxisorp surface, Nunc, Denmark) were coated with 100 µl of 0.5 g/l of *Str. albus* and 1.2 g/l of *A. versicolor* or a combination antigen dilution in PBS and by incubating at 4°C overnight. Plates were washed three times with PBS-Tween20 (Sigma Aldrich, Missouri, USA); 2.5% dilution of Tween20 in PBS) and once with the PBS. All plates were blocked with 200 µl of 1% bovine serum albumin (BSA; Sigma Aldrich, Missouri, USA) for 2 h at 37°C. The BSA was made in PBS. Plates were washed three times with PBS-Tween20) and once with the PBS. Microtiter plates were dried in hood cabin at ambient temperature and were then stored at -20°C covered with parafilm.

The serum sample was diluted in PBS, and 100 µl/well was pipetted to the coated plates. The used patient serum dilutions (in PBS) were discovered in the preliminary measurements and were used as follows:

IgA: 2.5% serum

IgG: 0.01% serum

IgG1: 12.5% serum

IgG3: 50% serum

IgM: 1% serum

The samples of four reference serums were measured in triplicate wells and the actual sample serum as duplicate wells. Serum samples were incubated at 37°C for one hour and washed three times with PBS-Tween 20.

The used secondary antibodies with a horseradish peroxidase (HRP) conjugate dilutions (in PBS) were discovered in the preliminary measurements and were used as follows:

Goat-anti-Human **IgA**-HRP (Thermo Fisher, Massachusetts, USA): 1/5,000 stock solution

Goat-anti-Human **IgG**-HRP (Thermo Fisher): 1/4,000 stock solution

Mouse-anti-human **IgG1**-HRP (Molecular Probes, Oregon, USA): 1/200 stock solution (200 µg powder + 500 µl H_2O [20% glycerol])

Mouse-anti-human **IgG3**-HRP (Thermo Fisher): 1/1,000 stock solution

Chicken-anti-human **IgM**-HRP (Thermo Fisher):1/10,000 stock solution

and 100 µl/well of a secondary antibody listed above was pipetted on the plates.

Secondary antibodies were incubated one hour at room temperature and washed three times with PBS-Tween 20 and once with PBS.

ABTS (Thermo Fisher, Novex) (1%; 2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt; this water-soluble HRP substrate yields a green end-product upon reaction with peroxidase) was added to citrate buffer (pH 4.2), the reaction was started by adding 0.1% H_2O_2 (Sigma) and then incubated 45 min at ambient temperature. The enzyme activity from the solid phase was related to the bound antibody, and the absorbance was measured at 415 nm using a Hidex Sense plate reader (Hidex, Turku,

Finland). The means of absorbance values for sample duplicates, reference triplicates and standard deviation (SD) for reference triplicates were calculated.

10.6 Data Handling

Raw data was handled and analysed by using Excel, Version 2016 (Microsoft, Redmond, Washington, USA).

Statistical analysis was made with SPSS version 25 (IBM, New York, USA).

The data normality was tested using Shapiro–Wilk test and due to the nonparametric distribution of the data, the significance of the data was valued by using the Mann-Whitney U test for calculating the p–values.

Graphs were prepared with Origin, Version 2016 (Microcal, OriginLab, Massachusetts, USA).

11. Results

The development of the spore-specific ELISA system was started by selecting four test serums from individuals outside the actual study material and by cultivating the spores of the chosen antigen species *S. albus* and *A. versicolor*. The repeatability and the deviation of the results were tested by multiple repeats and by comparing the results between different variations, different coating patches and reagent concentrations. The background signal measured from the 10 wells had an average OD reading of 0.0029 ± 0.00057, and the lowest reliable detection limit was computed by multiplying the gained standard deviation by 10 times being OD = 0.006, after the background signal was subtracted[39] . The upper range of the reliable detection limit was determined first by

calculating the average standard deviation (7.7%) from the four-person reference samples (Figure 1). This average was then subtracted from the OD = 3.999 (the maximum limit of the plate reader was OD = 4.000), and the highest reliable upper limit of the reliable detection was OD = 3.690. The reliable measurement range was from OD = 0.006 to OD = 3.690. The repeatability of the results was best within the same batch of coated plates, and 90 plates were prepared simultaneously in order to be able use the same patch of plates in actual sample measurements. Four selected reference serums, collected from the healthy volunteers from outside the actual study material (Table 1 and 2), were used to measure triplicate samples of every antigen and immunoglobulin class, and this was repeated three times (Figure 1). The repeatability was consistent, and the deviations acceptable (average = 7.7%). Because we were not able to use the existing reference measurements in order to gain reference data of the absolute Ig levels in our samples and since we did not have the Ig protein standards, we decided to compare the damaged and non-damaged buildings inside every Ig/antigen combination as shown in Figures 2 and 3. The aim was to detect possible differences between the Ig levels of individuals from damaged and non-damaged buildings.

Our first study material (Table 1) contained 70 serum samples from the workers in three microbe-damaged buildings (3 buildings, n = 70) and 89 serum samples from the workers from four non-damaged buildings. 4 buildings (n = 89) were tested by using *S. albus*, *A. versicolor* and combination of these spores as a solid phase combined antigen. IgA, IgG and IgM main class specific serum immunoglobulins were assayed. As shown in Figure 2, the serum samples from the microbe-damaged buildings had elevated IgG values against all used antigens compared with the serum samples from the reference buildings.

The differences when compared with the samples from the reference buildings, were significant ($p < 0.05$). No differences were observed when IgA and IgM were assayed.

The second material (Table 2) contained 43 serum samples from one damaged building (1 damaged building, n = 43) and 59 serum samples from the workers from one non-damaged building (1 non-damaged building, n = 59). Combined antigen was used to evaluate the levels of IgA, IgG, IgG1 and IgG3.

The values of IgG ($p < 0.05$), IgG1 ($p < 0.05$) and IgG3 ($p < 0.05$) from the workers from the microbe-damaged buildings were significantly higher than the samples from the non-damaged buildings.

The data had a non-parametric distribution, and the significance was valued by using the Mann-Whitney U test for calculating the p–values.

12. Discussion

Our results demonstrate that IgG values against the selected antigens were higher in group level in damaged buildings compared with those in the non-damaged buildings, and the differences were significant. Also, the combination antigen seemed to work, and in future research, this aspect will be more thoroughly studied, and more than two species will be tested to assess the responses. When using the combination antigen, the amount of individual antigen was halved, and the results were not directly comparable between one antigen and the combined antigen. Nevertheless, if the levels were high with an individual antigen[18] , then the same trend was observed with combined antigen (data not presented).

The antigens were chosen according to the microbes found from the damaged sites, and *S. albus* and *A. versicolor* are both considered as the indicator species of the severe microbe damage frequently found in the moisture-damaged buildings in Finland[40] .

The spore cultivation and harvesting was operated according to the standard protocol presented by the Kieser et al.[37]. After the filtration, in microscope, suspensions were very clearly consisting of spores, but it is not impossible that small fragments of mycelium was also present, especially when cultivating *A. versicolor* on the rich MA-agar. The MS agar is commonly used for the spore cultivation and harvesting in *Streptomyces* species.

Activation of the immune system is a normal defence reaction against the threats caused by invading microbes, and the raised Ig levels are not necessarily the markers of an anomaly or disease. According to the health status and symptom questionnaire, the levels of individuals with high Ig levels and the severe symptoms caused by the microbe-damaged building did not significantly correlate. Still, a raised serum IgG level seems to be a marker of the microbe damage to a certain degree in the building. IgG correlates with exposure and not with symptoms, which has been shown previously among workers with similar occupations[20] .

There are existing serological microbe ELISA methods[41] , but these do not specifically assess spore-specific responses. There is a difference in the antigen structure between the a viable microbe cell and dry, dormant and dead microbe material like spores and mycelium. Because the exposure is most probably caused by the latter material[42] , there is a greater possibility of bias in the antibody assessment using antigens prepared from the cultivated and viable microbes.

One starting point of the study was to evaluate whether the exposure could be found using only one "common" antigen. The spore surface molecular structure is reported to be conserved[43], suggesting that there could be enough cross-reactivity. However, there was not a strong enough correlation, when we compared the results made with the *S. albus* and *A. versicolor* (data not shown), and this hypothesis was not pursued.

How do the Ig levels assessed from the serum sample reflect the hypothesis that the exposure is originating in the respiratory track? The mucosal surfaces of lungs and other respiratory tracks are in direct continuity with the external environment and are therefore a major site of microbe exposure. The huge external surface that bathe the whole respiratory track form a unique immunologic system involved in host defence designated as bronchus-associated lymphoid tissue (BALT)[21, 44, 45] . Dysfunctions and unbalances can result in chronic pulmonary infections, allergic disease or immunopathology in lung tissue[44] . The lung is an important immunological organ containing lymphoid tissue and thus houses a remarkable arsenal of Igs. These Igs have a major task to defend against antigens by performing the neutralization and removal of the target as part of the cellular arm of the immune system[44-47] . Plasma cells have been detected in bronchial mucosa and in the *lamina propria* in airways indicating a local production of the Igs from lung-associated lymph nodes (LALNs)[47, 48] . The diffusion from the vascular compartment is another considerable source of the lung Igs and vice versa. All Ig classes and most of their subclasses are known to be produced in the lung tissue[44] .

IgM is the primary Ig secreted after the activation of B lymphocytes to the formation of plasma cells, and IgM antibodies have a lower specificity when compared to the

specificity of IgG and IgA. The soluble form of IgM is mostly pentameric, having ten antibody binding sites and is thought to be an effective complement system activator and opsonin[21] . Our results showed no significant differences between the spore-specific IgM from damaged and non-damaged building users. There probably was no primary state activation in progress or if activation occurred, it was located in the lung tissue causing restriction of the diffusion of a large pentameric protein to the plasma.

Dimeric IgA is mainly produced by the mucous membranes, and no significant differences were observed in the serum samples between the damaged and reference building users. In blood and in the lungs, the main subclass is IgA1, but in lung tissue, the proportion of secretory IgA2 is higher compared to the blood[44, 47, 49] . This different composition of IgA indicates that the local production occurs in lung tissue in addition to the diffusion of the IgA from blood. Apparently, the assessment of the IgA levels related to the respiratory track exposure is not plausible from the serum sample.

The predominant Ig class in plasma, IgG, has four described subclasses designated as IgG1, IgG2, IgG3 and IgG4, all of which have also been detected in the bronchoalveolar lavage fluid samples from healthy individuals[20, 50, 51] . In this assay, only IgG1 and IgG3 subclasses were studied, and both of these were elevated in serum samples from the damaged buildings. These subclasses were chosen to be analysed first because of their role in phagocytosis, but in the future, especially the IgG2 subclass will also be investigated.

It has been reported that IgG3- and IgG4-secreting plasma cells are relatively more frequent in the bronchial mucosa than those of IgG1 and IgG2 indicating that isotypes of IgG3 and IgG4 are mostly produced locally in lung tissue at least to a much greater degree than IgG1 and IgG2, which are supposed to be mostly plasma-related[52-54] .

13. Conclusions

The antigens derived from the microbe-damaged buildings have an impact on the human immune system by elevating the total IgG and IgG1 and IgG3 levels in building users. This effect can be measured with ELISA by using the dormant material, like spores and the spore mixtures as an antigen. In our future studies, we will focus on the spore-antigen structure and the cross-reactivity of these antigens. From an immunological research perspective, we are interested in the absolute levels of the serum Igs and all IgG subclasses including IgG2 and IgG4.

14. Appendices

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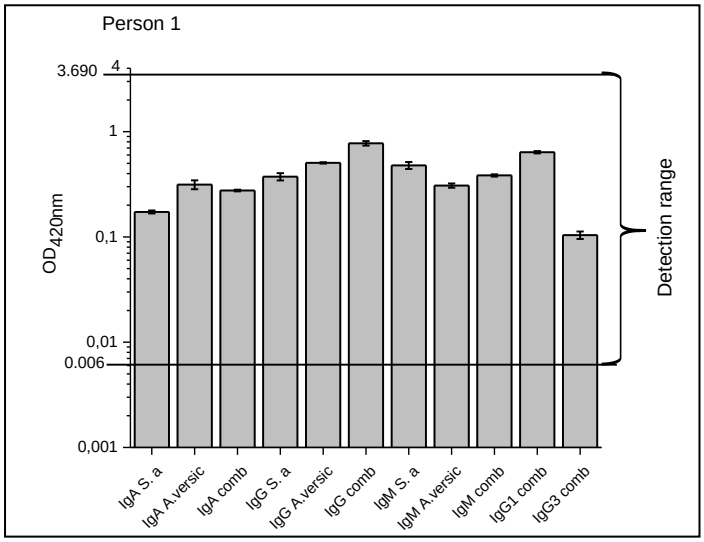
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4
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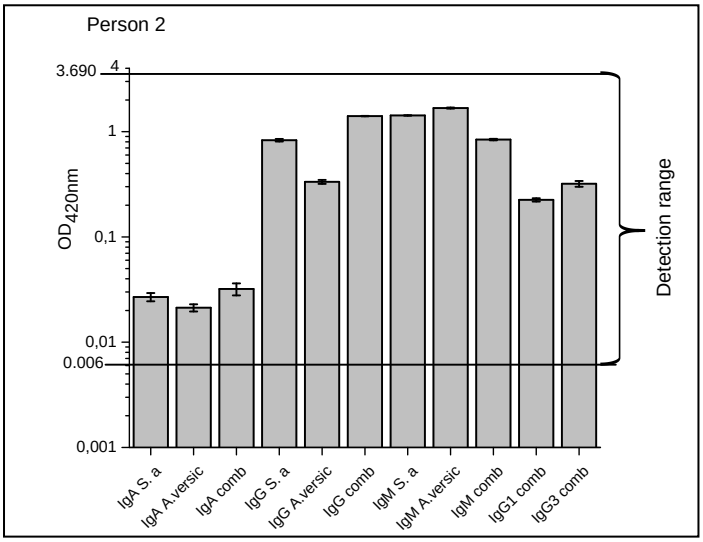
0,1
0,01
0.006
0,001
OD420nm
Detection range
IgA S. a
IgA A.versic
IgA comb
IgG S. a
IgG A.versic
IgG comb
IgM S. a
IgM A.versic
IgM comb
IgG1 comb
IgG3 comb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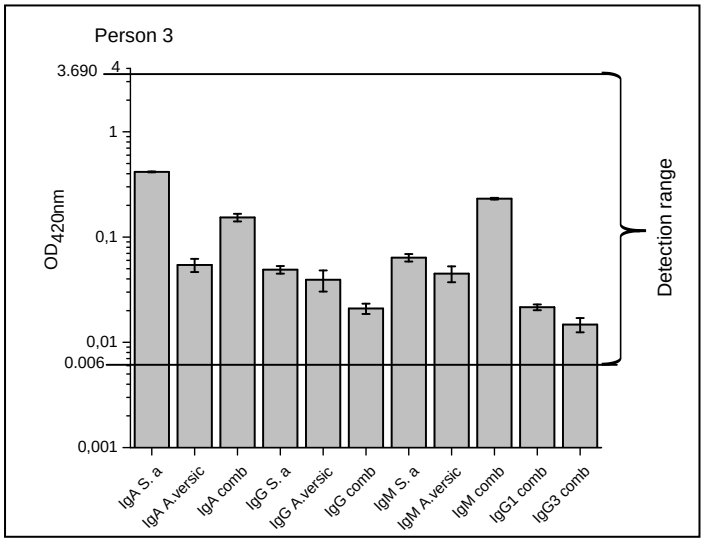
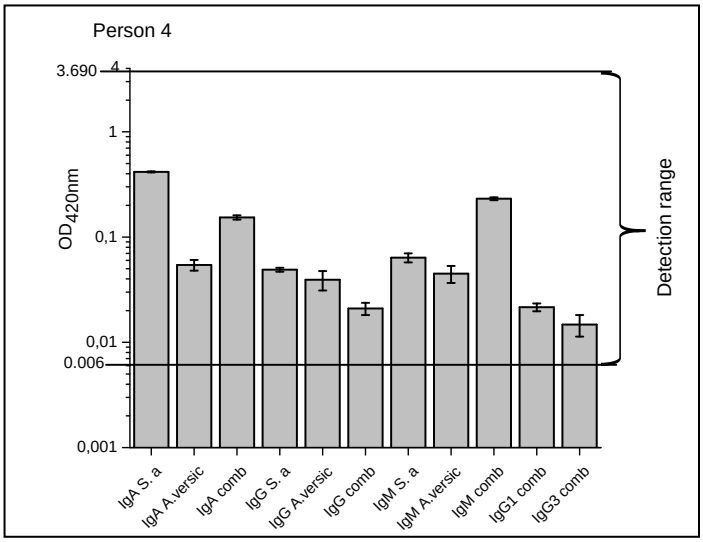
3.690
4
1
0,1
0,01
0.006
0,001
OD420nm
Detection range
IgA S. a
IgA A.versic
IgA comb
IgG S. a
IgG A.versic
IgG comb
IgM S. a
IgM A.versic
IgM comb
IgG1 comb
IgG3 comb


Person 3
3.690
4
1
0,1
0,01
0.006
0,001
OD420nm
Detection range
IgA S. a
IgA A.versic
IgA comb
IgG S. a
IgG A.versic
IgG comb
IgM S. a
IgM A.versic
IgM comb
IgG1 comb
IgG3 comb


Person 4
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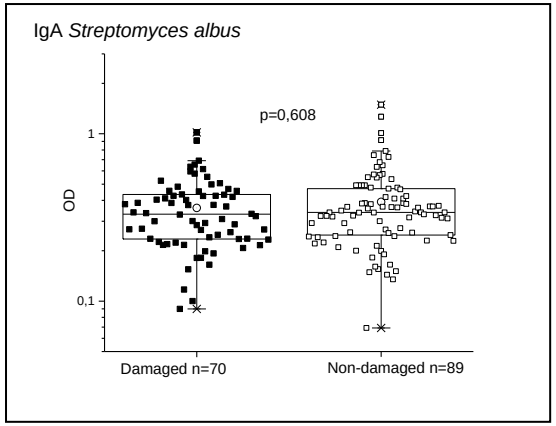
4
1
0,1
0,01
0.006
0,001
OD420nm
Detection range
IgA S. a
IgA A.versic
IgA comb
IgG S. a
IgG A.versic
IgG comb
IgM S. a
IgM A.versic
IgM comb
IgG1 comb
IgG3 comb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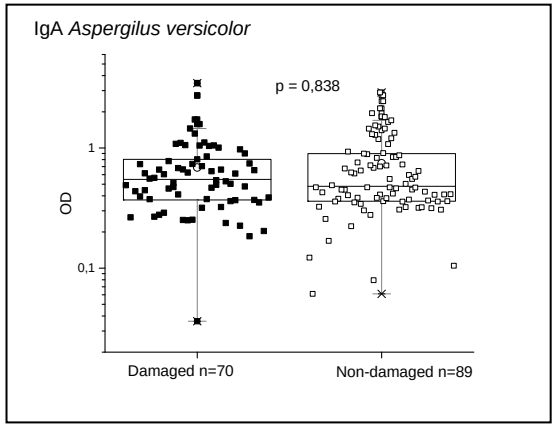
0,1
Damaged n=70
Non-damaged n=89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p = 0,838
OD
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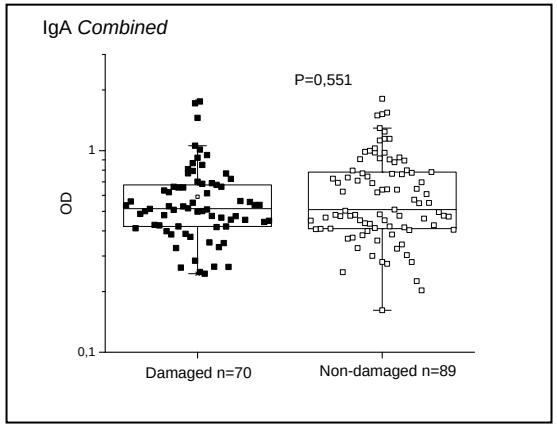
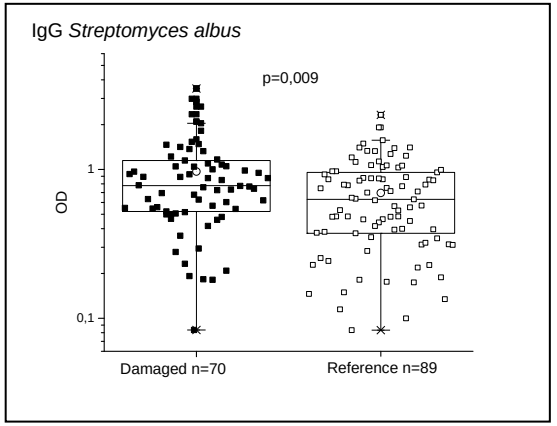
0,1
Damaged n=70
Non-damaged n=89


IgA Combined
P=0,551
OD
1
0,1
Damaged n=70
Non-damaged n=89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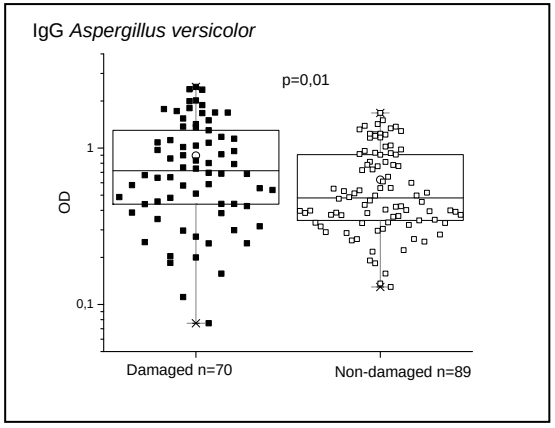
p=0,009
OD
1
0,1
Damaged n=70
Reference n=89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OD
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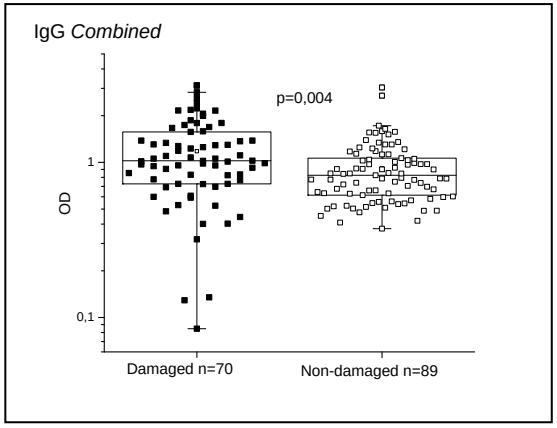
0,1
Damaged n=70
Non-damaged n=89


IgG Combined
p=0,004
OD
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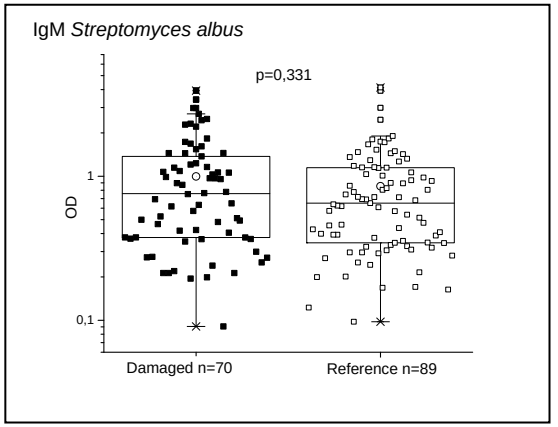
0,1
Damaged n=70
Non-damaged n=89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OD
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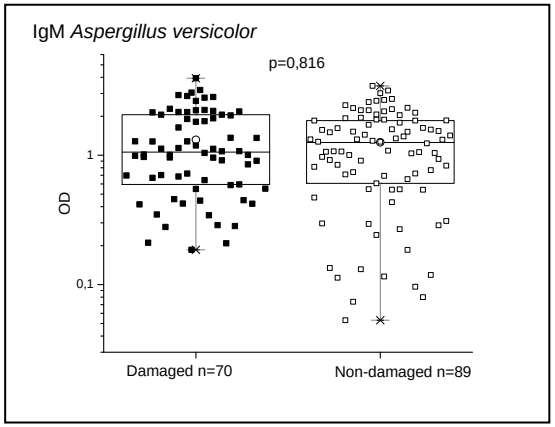
0,1
Damaged n=70
Reference n=89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0,1
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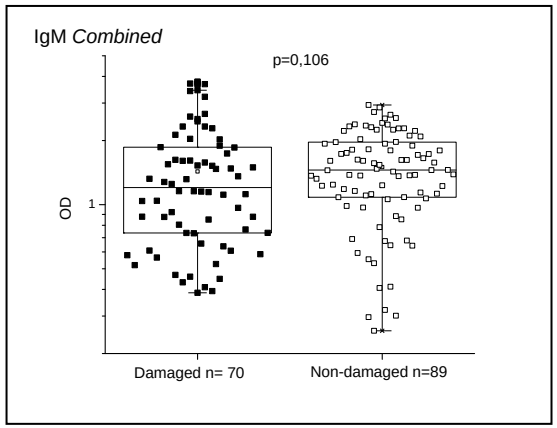
Non-damaged n=89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p=0,106
OD
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Damaged n= 70
Non-damaged n=89

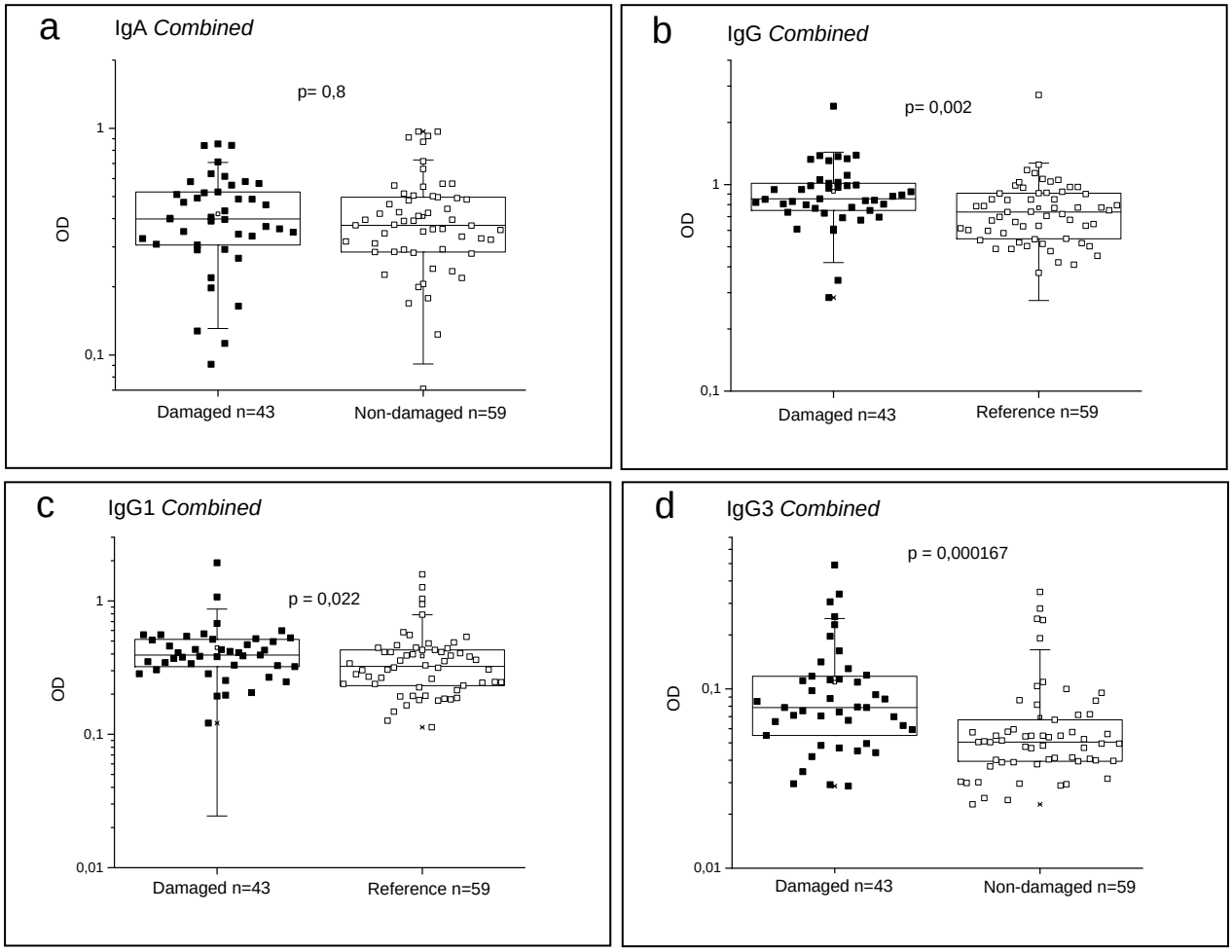
a
IgA *Combined*
p= 0,8
OD
1
0,1
Damaged n=43
Non-damaged n=59
b
IgG *Combined*
p= 0,002
OD
1
0,1
Damaged n=43
Reference n=59
c
IgG1 *Combined*
p = 0,022
OD
1
0,1
0,01
Damaged n=43
Reference n=59
d
IgG3 *Combined*
p = 0,000167
OD
0,1
0,01
Damaged n=43
Non-damaged n=59

Figure Captions

Figure 1: Four reference samples: The results of the four reference samples outside the actual test material. Results are shown as an average and STDV from the triplicate measurements. The reliable measure range (OD = 0.006 – OD = 3.690) is presented with black lines.

Figure 2: IgA, IgG and IgM: Spore-specific IgA (upper row), IgG (middle row) and IgM (lower row) against *S. albus*, *A. versicolor* and combined antigen. Statistic is form 25%, 75%, the line is median, whiskers are outliers, and circles represent the average and crosses min and max. P-value was calculated by using the Mann-Whitney U test.

Figure 3: IgA, IgG, IgG1 and IgG3 and combined antigen: Spore-specific a) IgA, b) IgG, c) IgG1 and c) IgG3 against and combined (*S. albus*, *A. versicolor*) antigen. Statistic is form 25%, 75%, the line is median, whiskers are outliers, and circles represent the average and crosses min and max. P-value was calculated by using the Mann-Whitney U test

Tables and captions

Table 1: The first study material. Microbe damages were estimated from the material samples collected from the target buildings and analysed by the Aerobiology Unit of the University of Turku using the plate-counting colony forming unit method.

Damage classification: 0/*** (no damage), */*** (mild), **/*** (damaged) and ***/*** (severely damaged)

Study material 1	
Microbe-damaged buildings	Serum samples
Health care centre 1, Eastern Finland S. albus ***/*** A. versicolor **/***	26
Apartment Building (block of flats), Southern Finland S. albus ***/*** A. versicolor ***/***	18
Bedroom and Kitchen, Fire station 1, Southern Finland S. albus **/*** A. versicolor **/***	26
	n=70
Non-damaged buildings	Serum samples
Bedroom and Kitchen, Fire station 2, Southern Finland S. albus 0/*** A. versicolor 0/***	11
Bedroom and Kitchen, Fire station 3, Southern Finland S. albus 0/*** A. versicolor 0/***	12
Bedroom and Kitchen, Fire station 4, Southern Finland S. albus 0/*** A. versicolor 0/***	7
School 1, Southern Finland S. albus 0/*** A. versicolor 0/***	59
	n=89

Table 2: The second study material. Microbe damages were estimated from the material samples collected from the target buildings and analysed by the Aerobiology Unit of the University of Turku using the plate-counting colony forming unit method.
Damage classification: 0/*** (no damage), */*** (mild), **/*** (damaged) and ***/*** (severely damaged)

Study material 2	
Microbe-damaged buildings	Serum samples
Health care centre 1, Eastern Finland S. albus ***/*** A. versicolor **/***	n=43 (26 like in 1. Study material + additional 17 serums samples)
Non-damaged buildings	Serum samples
School 1, Southern Finland S. albus 0/*** A. versicolor 0/***	n=59