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# Passive Airborne Dust Sampling with the Electrostatic Dustfall Collector: Optimization of Storage and Extraction Procedures for Endotoxin and Glucan Measurement

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**We recently introduced a passive dust sampling method for airborne endotoxin and glucan exposure assessment—the electrostatic dustfall collector (EDC). In this study, we assessed the effects of different storage and extraction procedures on measured endotoxin and glucan levels, using 12 parallel EDC samples from 10 low exposed indoor environments. Additionally, we compared 2- and 4-week sampling with the prospect of reaching higher dust yields. Endotoxin concentrations were highest after extraction with pyrogen-free water (pf water) + Tween. Phosphate-buffered saline (PBS)–Tween yielded significantly (44%) lower levels, and practically no endotoxin was detected after extraction in pf water without Tween. Glucan levels were highest after extraction in PBS–Tween at 120°C, whereas extracts made in NaOH at room temperature or 120°C were completely negative. Direct extraction from the EDC cloth or sequential extraction after a preceding endotoxin extraction yielded comparable glucan levels. Sample storage at different temperatures before extraction did not affect endotoxin and glucan concentrations. Doubling the sampling duration yielded similar endotoxin and only 50% higher glucan levels. In conclusion, of the tested variables, the extraction medium was the predominant factor affecting endotoxin and glucan yields.**

*Keywords:* dust sampling; endotoxin; exposure assessment; exposure assessment methodology; glucans; indoor environment; lipopolysaccharide; passive sampling

## INTRODUCTION

The electrostatic dustfall collector (EDC) has recently been proposed as a sampler that can be applied in population studies in the indoor environment for airborne dust glucan (Noss *et al.*, 2009). Since the introduction, it has found a first application in airborne endotoxin assessment in the low exposure indoor home environment (Noss *et al.*, 2008), but it can also be used in high-exposure occupational environments for both endotoxin and allergen measurements (Samadi *et al.*, 2010) or assessment of exposures to bacteria and fungi in houses of farmers and stables and barns (Normand *et al.*, 2009). This new passive airborne dust sampling method has in several different studies proven its usefulness as a tool to assess exposure to airborne dust and microbial constituents in home or work environments. It is easy to use for the participants of a study, can be sent by mail, and is a cheap and reliable method to collect airborne dust on a large scale.

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We have introduced the EDC employing extraction and analytical techniques that were developed for floor or mattress dust samples (Douwes *et al.*, 1996; Schram-Bijkerk *et al.*, 2005). However, since the electrostatic cloth material on which dust is collected differs from the nylon socks or filters used for floor or mattress dust, the EDC sample processing may not be the optimal procedure.

Several studies have shown that factors like the type of filter or extraction buffer can influence the efficiency of endotoxin extraction. Enhanced extraction efficiency has been reported for glass fiber filters (Olenchock *et al.*, 1989), which can be even more increased by adding 0.05% Tween to the extraction medium (Douwes *et al.*, 1995; Liebers *et al.*, 2007; Spaan *et al.*, 2007; Spaan *et al.*, 2008). Additionally, factors influencing the endotoxin activity in the *Limulus* Amebocyte Lysate (LAL) assay have been found to depend on the environmental source of the samples (Reynolds *et al.*, 2002; Reynolds *et al.*, 2005). For floor or mattress dust samples, such comparison and optimization studies have hardly been reported, even though it may be anticipated that different sampling materials and procedures may affect the extraction efficiency. Studies on the influence of such factors on the analysis for glucan exposure assessment have not been published to our knowledge. It is known that solubility varies for different glucans, depending on the solvent (aqueous, alkaline, or dimethyl sulfoxide) and procedure [room temperature (RT) or heated] (Stone and Clarke, 1992; Noss *et al.*, 2009). Thus, it can be expected that the use of different extraction procedures will influence the glucan yields.

The aim of this study was therefore to assess the effects of different procedures for endotoxin and glucan analysis using EDC samples. We collected parallel samples to study the effects of storage at RT versus −20°C before extraction and the influence of different extraction media and procedures. For glucan analysis, we also compared yields after extraction directly from the cloths or after preceding endotoxin extraction. Since sampling with the EDC collects generally low dust amounts, we further compared 2 weeks with 4 weeks sampling time to explore the prospect of reaching higher dust yields by longer sampling durations.

## EXPERIMENTAL

### *Study design*

*Parallel sampling study.* In 10 homes, three parallel EDC samplers were used for 14 days. With each EDC, settling dust is collected on four electrostatic cloths mounted in a 40 × 30 cm plastic folder. The cloths were rendered pyrogen free (pf) before use by heating overnight at 200°C. The EDC was left for 14 days in horizontal position with each cloth (area: 0.0209 $m^2$) exposed to the air (Noss *et al.*, 2008). We thus collected 3 × 4 parallel cloth samples per home. We treated three EDCs (field blanks) parallel to the home samples, but opened them only shortly. The 12 samples from each home and the field blanks were randomly assigned to a combination of storage and extraction procedures such that one cloth per home was assigned for each combination (Fig. 1). After the assigned extraction procedure, aliquots were stored for ∼5 weeks at −20°C before analysis.

*Sampling duration study.* Seven students sampled their homes with one EDC for 2 weeks and one EDC for 4 weeks. Starting day of a sampling was identical for both EDCs per home. Samples were stored prior to extraction at RT for maximally 2 weeks. All extracts were stored for ∼5 weeks at −20°C.

### *Extraction*

*Endotoxin.* The extraction procedure was adapted from a previous study (Noss *et al.*, 2008). Briefly, endotoxin was extracted from the electrostatic cloths by shaking for 1 h at RT in either 20 ml pyrogen-free water (pf water), pf water plus 0.05% Tween-20 (pf water + Tween), or phosphate-buffered saline (PBS) plus 0.05% Tween-20 (PBS + Tween). From the extracts, 10% of the extraction volume (2 ml) was harvested, centrifuged at 1000 g for 15 min and supernatants were stored as aliquots at −20°C until analysis ∼5 weeks later.

Cloths from the sampling duration study were extracted as described previously (Noss *et al.*, 2008) with pf water + Tween as the extraction solution.

*Glucan.* Samples of the parallel study that had been extracted for endotoxins in pf water + Tween were subsequently extracted for glucans (Schram-Bijkerk *et al.*, 2006; Noss *et al.*, 2009). Briefly, the harvested 10% extraction volume was replaced by 10× concentrated PBS, resulting in a final 1× PBS + Tween solution. To samples assigned to the direct extraction procedure, 20 ml of the appropriate extraction medium (PBS + Tween or 0.05 M NaOH) was added. For extraction in 0.05 M NaOH at RT, cloths were shaken for 1 h at RT; ∼9 ml extraction fluid was collected and centrifuged at 1000 g for 15 min after which the supernatant was harvested. For the extractions with autoclaving, cloths were shaken for 15 min, heated for 1 h at 120°C (Douwes *et al.*, 1996; Schram-Bijkerk *et al.*, 2006), cooled down, and shaken for another 15 min. Then ∼9 ml supernatant was collected and centrifuged at 1000 g for 15 min.

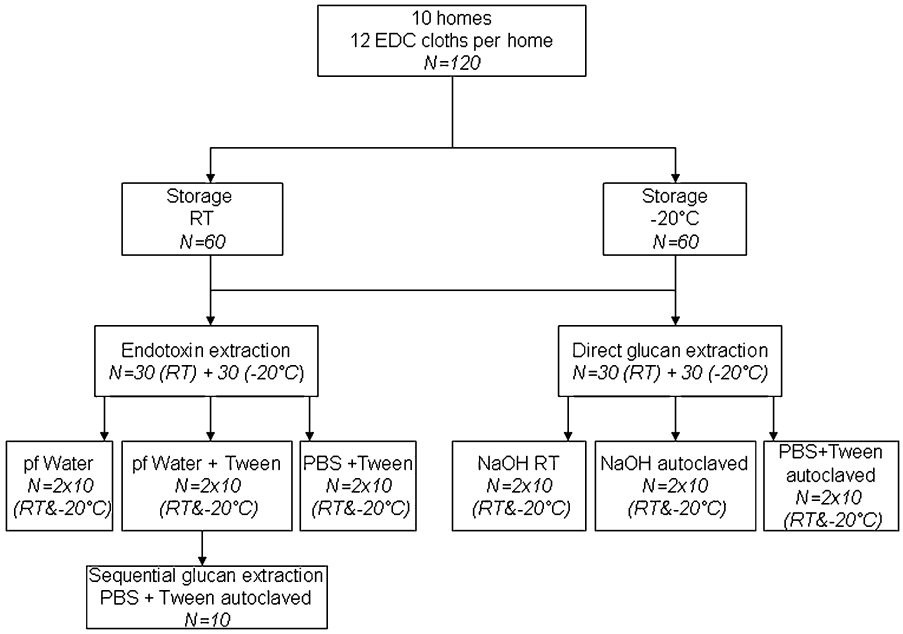


**Fig. 1.** Schematic overview of the design of the experiment, the storage conditions before extraction, extraction buffers, and procedures are denoted.

Supernatants were stored as 1 ml aliquots at −20°C. Glucans were analyzed after ∼5 weeks of storage.

Cloths from the sampling duration study were extracted for glucans after endotoxin extraction. The extraction was performed by autoclaving in PBS + Tween (Schram-Bijkerk *et al.*, 2006; Noss *et al.*, 2009).

### *Analysis*

*Endotoxin.* Endotoxin analysis was performed as described previously (Noss *et al.*, 2008). Each single extract was diluted 50 times in pf water and analyzed in the quantitative kinetic chromogenic LAL assay (Lonza, Walkersville, MD, USA; LAL-Lysate lot GL155U; standard *Escherichia coli* O55:B5 lot GL1157; reference standard endotoxin:control standard endotoxin ratio, 14 EU $ng^{-1}$). The cut-off signal ($v_{max}$) of the kinetic LAL Assay was defined as the average of the assay blanks plus two times the standard deviation of these blanks. The corresponding sensitivity of the assay [limit of detection (LOD)] for an undiluted sample was 0.009 EU $ml^{-1}$ and thus for the dilution used in this study (1/50) the LOD was 0.45 EU $ml^{-1}$.

*Glucans.* Glucan measurements were performed with the previously described U1B2 β-(1,3)-glucan sandwich enzyme immunoassay (EIA) (Noss *et al.*, 2009). Briefly, microtiter plates were coated with the polyclonal rabbit anti-β-(1,3)-glucan antibody (U1). Binding of the biotinylated monoclonal mouse anti-β-(1,3)-glucan antibody (B2) was quantified with Poly-horseradish peroxidase Streptavidin. As calibration standard, we used baker's yeast glucan. Samples were incubated in four 2-fold dilutions in PBS–Tween. All 0.05 M NaOH extracts were adjusted to a pH 7.0 prior to analysis with 2 M hydrochloric acid. Glucan extracts of all EDC cloths were tested in dilutions of 1/5, 1/10, 1/20, and 1/40 in the glucan sandwich assay. The LOD was defined as previously (Noss *et al.*, 2009) and for a 1/5 diluted sample it was 4.2 ng $ml^{-1}$.

### *Statistical analysis*

Samples with signals below the cut-off were assigned 2/3 of the LOD for both endotoxin and glucan measurements. Since data were log-normally distributed, statistical analyses were performed using ln-transformed values. To investigate the influence of different variables on endotoxin and glucan concentrations, mixed-effects models were applied using home ID as random factor to correct for possible correlation for cloths from the same home. Temperature of storage before extraction, extraction medium, or sampling duration—according to the study and agent—were introduced as fixed factors. Statistical analyses were performed using SAS software v 9.1.3 (SAS Institute Inc., Cary, NC, USA). Graphs were produced with Graphpad Prism 4.0 (Graphpad Software Inc, San Diego, CA, USA).

## RESULTS

### *Parallel sampling study*

*Endotoxins.* Cloths were stored at RT or −20°C before extraction and extracted in pf water, pf water + Tween, or PBS + Tween. We analyzed 10 cloths for each combination of storage temperature and extraction medium (Fig. 2). Only one of the cloths extracted in pf water yielded an endotoxin concentration above the LOD. Therefore, the results of the pf water extracts were not included in further statistical analysis. One cloth extracted in pf water + Tween and six cloths extracted in PBS + Tween showed endotoxin activity below the cut-off level. Mean endotoxin concentrations after pf water + Tween extraction were higher than after PBS + Tween extraction, while the temperature at which EDC cloths were stored before extraction did not seem to influence the results.

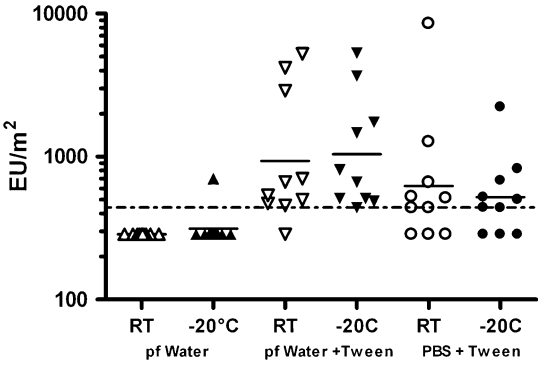


**Fig. 2.** Endotoxin concentrations in endotoxin unit per square meter measured in cloths stored at RT or −20°C before extraction in either pf water, pf water + Tween, or PBS + Tween; the dotted line denotes the LOD and the horizontal bars denote the GMs for each group.

Figure 3 illustrates more clearly that the difference between endotoxin levels measured in pf water + Tween and PBS + Tween extracts was most pronounced at higher concentrations. Correlations between endotoxin levels on cloths extracted in pf water + Tween and on cloths extracted in PBS + Tween were high for both storage at −20°C ($r = 0.88$) and—after removal of one outlier—also for storage at RT ($r = 0.84$). Concordantly, levels of cloths stored at different temperatures before the extraction correlated strongly ($r = 0.93$) when extracted in pf water + Tween and—after removing the same outlier—also for samples extracted in PBS + Tween ($r = 0.99$).

Mixed-effects regression analysis was applied to statistically evaluate the effects of storage at different temperatures before extraction and the use of different extraction media on the measured endotoxin concentrations (Table 1).

The analysis confirmed previous results, namely that storing cloths at −20°C before the extraction results in similar levels as storing cloths at RT, while extracting cloths in PBS + Tween leads to

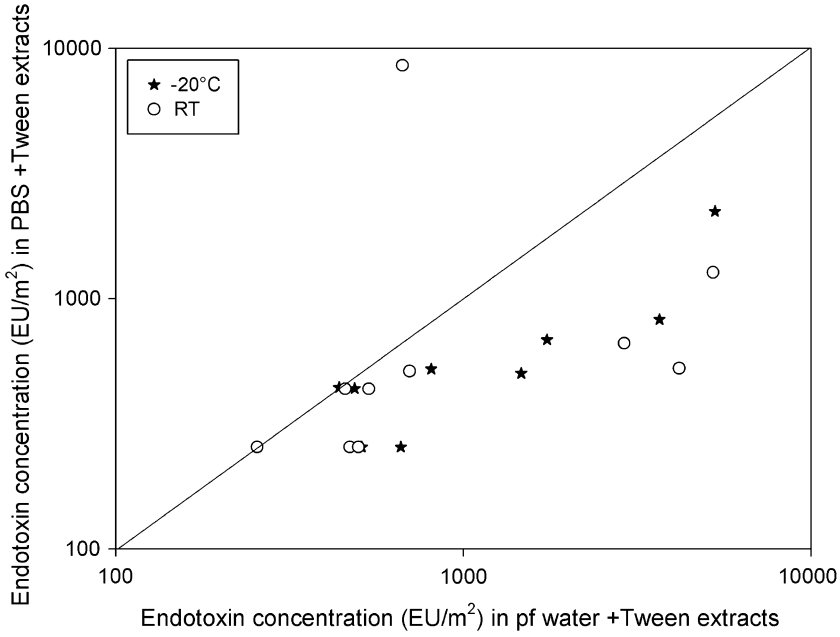


**Fig. 3.** Comparison of endotoxin levels of cloths extracted either with pf water + Tween or PBS + Tween for both storage temperatures before extraction.

Table 1. Effect ($e^{\beta}$) and 95% confidence interval (CI $e^{\beta}$) of storing cloth at −20°C before extraction and of PBS+ Tween as the extraction medium on the endotoxin load (EU $m^{-2}$) measured on parallel study cloths relative to storing cloths at RT and extraction in pf water + Tween

| | Endotoxin |
|---|---|
| Intercept $e^{\alpha}$ (CI $e^{\alpha}$) | 986 (0.55–1.94) |
| Storage before extraction | |
| −20°C $e^{\beta}$ (CI $e^{\beta}$) | 0.97 (0.65–1.45) |
| RT $e^{\beta}$ | 1.00 |
| Extraction medium | |
| PBS + Tween $e^{\beta}$ (CI $e^{\beta}$) | 0.56 (0.38–0.84) |
| pf water + Tween $e^{\beta}$ | 1.00 |

significantly 44% lower endotoxin concentrations than extraction in pf water + Tween (Table 1).

*Glucans.* Similar to the endotoxin measurements, cloths were analyzed for β-(1,3)-glucan concentrations for each combination of storage temperature and extraction medium. Cloths were extracted in NaOH—at RT or autoclaved—or autoclaved in PBS+ Tween—either after previous endotoxin extraction (sequential procedure) or with no prior extraction step. Only one of the cloths extracted in NaOH yielded a measurable glucan concentration (Fig. 4), and we therefore did not include the results of NaOH extraction in further statistical analysis.

Sequentially extracted cloths showed on average slightly higher glucan levels than directly extracted cloths. Levels measured in cloths stored frozen as compared to storage at RT before the extraction were similar for both sequentially and directly extracted cloths (Fig. 4).

The Pearson correlation was moderate to strong ($r > 0.7$) between the glucan concentrations measured on cloths stored at RT or at −20°C, as well as between glucan concentrations measured in extracts after either sequential or direct extraction.

As for the endotoxin measurements, mixed-effects regression analysis was applied to statistically evaluate the effects of storage and use of different extraction media and procedures on the glucan concentrations measured (Table 2).

Storing cloths at −20°C compared to storing at RT before extraction resulted in (non-significant) 10% higher glucan concentration, while direct extraction resulted in (just-significant) 11% lower glucan concentrations when compared to sequential extraction.

*Sampling duration study*

We collected dust in seven homes with two EDC samplers, one exposed for 2 weeks and the other for 4 weeks. The coefficient of variance (CV%) between

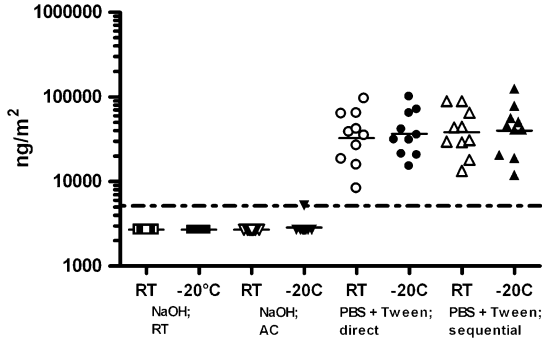


**Fig. 4.** Glucan concentrations in nanogram per square meter measured in cloths after storage at RT or −20°C before extraction in either NaOH at RT, NaOH with autoclaving (AC), PBS + Tween AC directly, or PBS + Tween AC after the endotoxin extraction; dotted line denotes LOD; horizontal bars denote GMs.

Table 2. Effect ($e^{\beta}$) and 95% confidence interval (CI $e^{\beta}$) of storing cloth at −20°C before extraction and direct extraction on the glucan load (μg $m^{-2}$) measured on parallel study cloths relative to storing cloths at RT and sequential extraction

| | Glucan |
|---|---|
| Intercept $e^{\alpha}$ (CI $e^{\alpha}$) | 37.5 (24.4–63) |
| Storage before extraction | |
| −20°C $e^{\beta}$ (CI $e^{\beta}$) | 1.1 (0.97–1.22) |
| RT $e^{\beta}$ | 1.00 |
| Extraction procedure | |
| Direct $e^{\beta}$ (CI $e^{\beta}$) | 0.89 (0.79–0.996) |
| Sequential $e^{\beta}$ | 1.00 |

endotoxin levels on different cloths within the same EDC was on average 19% (range, 4–64%), and for glucan levels 15% (range, 7–23%). These results are suggesting a small sampling error and very similar outcomes for the four sampled cloths within one EDC. For each EDC, arithmetic mean endotoxin or glucan concentrations of the values measured in the four cloths were calculated and used in further analysis. Endotoxin and glucan concentrations were above LOD (Fig. 5) for all cloths.

Endotoxin concentrations were similar after 2 and 4 weeks sampling, while glucan concentrations were slightly higher after 4 weeks sampling when compared to 2 weeks sampling. Pearson correlation coefficients calculated between 2 and 4 weeks sampling were moderate for endotoxin ($r = 0.72$) and high for glucan measurements ($r = 0.95$). Mixed-model regression analysis showed that a 4 weeks sampling period led to (non-significant) 5% higher endotoxin concentrations and (significant) 51% higher glucan concentrations (Table 3).

## DISCUSSION

The aim of this study was to optimize endotoxin and glucan exposure assessment with the EDC. We investigated the effect of different sample storage conditions and extraction procedures. Storage of samples before extraction at either RT or −20°C resulted in similar endotoxin or glucan levels, independent of extraction solution or procedure. For endotoxins, this is in line with previously reported results by Spaan *et al.* (2007), who found similar levels in parallel samples—on either glass fiber or Teflon filters—stored for ∼1 week at −20 or 4°C. Bacterial growth has been reported to cause 20% higher endotoxin levels after long-term storage (several years) of half of a filter sample at 4°C when compared to the other half that had been analyzed within a short time (Morgenstern *et al.*, 2006). Microbial growth may occur more rapidly on samples from a humid or very contaminated environment, while for 'normal' dust samples short-term storage at RT is possible. For longer periods, however, and for such more humid 'risk' samples, storage at −20°C may be more reliable by preventing bacterial growth.

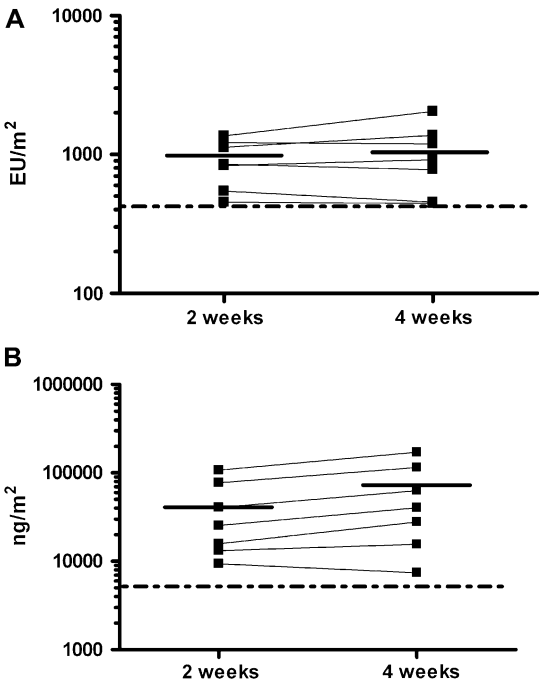


**Fig. 5.** Endotoxin (A) and Glucan (B) concentrations measured in cloth extracts after 2 weeks or 4 weeks of sampling; the dotted line denotes the LOD; the horizontal bars denote the GMs.

Table 3. Effect ($e^{\beta}$) and 95% confidence interval (CI) of sampling 4 weeks on the endotoxin (EU $m^{-2}$) and glucan (μg $m^{-2}$) loads measured in cloths relative to sampling 2 weeks

| | Endotoxin | Glucan |
|---|---|---|
| Intercept $e^{\alpha}$ (CI $e^{\alpha}$) | 823 (0.54–1.3) | 27.3 (11–73) |
| Sampling period | | |
| 4 weeks $e^{\beta}$ (CI $e^{\beta}$) | 1.05 (0.88–1.25) | 1.51 (1.4–1.63) |
| 2 weeks $e^{\beta}$ | 1.00 | 1.00 |

In our study, pf water as extraction solution did not yield endotoxin concentrations in the measurable range. Spaan *et al.* (Spaan *et al.*, 2007) and Liebers *et al.* (Liebers *et al.*, 2007) reported measurable endotoxin levels in pf water extracts, but they also found higher extraction efficiency with Tween in the extraction medium. Moreover, lower extractability could be caused by the sampling material. Douwes *et al.* (Douwes *et al.*, 1995) reported a lower endotoxin yield from airborne dust collected on cellulose filters and Bogdanovic *et al.* (Bogdanovic *et al.*, 2006) suggested that Tween may improve allergen release by increasing the wettability on hydrophobic filter surfaces. The electrostatic cloths in this study are highly electrostatic and made of hydrophobic polyester. Therefore, the suggestion of increased wettability by Tween and thus increased endotoxin release could apply in this study as well.

In the study of Spaan *et al.* (Spaan *et al.*, 2007), the geometric mean (GM) of airborne endotoxin was ∼3200 EU $m^{-3}$ when measured in pf water + Tween extracts—and 1500 EU $m^{-3}$ in pf water. From these data, the endotoxin load on the filters can be calculated as 16–64 × $10^5$ EU $m^{-2}$ as measured after extraction in pf water + Tween and 7.5–30 × $10^5$ EU $m^{-2}$ in pf water extracts (based on filter size and flow rate). Similar values can be derived from the data published by Liebers *et al.* (Liebers *et al.*, 2007). In our study, we found on average ∼800 EU $m^{-2}$ on the passively exposed EDC cloths after extraction in pf water + Tween—a >50–100 times lower load. Thus, our results seem compatible with their conclusion that the effect of Tween on extraction efficiency is more pronounced at lower loads of endotoxin on the sampling surface. An additional factor might be the above-mentioned influence of the cloth material—polyester—which may lead to lowered extractability in pf water.

The correlation between PBS + Tween and pf water + Tween extracts was strong, but PBS + Tween did yield significantly lower levels and even more so at higher endotoxin concentrations. Spaan *et al.* (Spaan *et al.*, 2008) reported that using a phosphate-containing buffer (triethylamine-phosphate buffer) might interfere with the reactivity of standard

endotoxin the LAL assay. Such interference may also explain the apparently lower concentrations measured in our PBS + Tween extracts, but lower extraction efficiency in PBS + Tween is another possible cause.

A potential weakness of the findings from this study is that our low endotoxin exposure levels cannot be generalized to studies with much higher endotoxin exposure. On the other hand, recent spiking experiments in one of our laboratories, in which 25 mg sieved house dust was sprinkled on EDC cloths confirmed that extraction in pf water + Tween worked well with non-significant difference in endotoxin yield from the cloths from the EDC versus from 25 mg of the dust not applied to the cloths (P. S. Thorne *et al.*, in preparation). This indicates that the release of higher concentrations of endotoxin from dust attached to the EDC cloths will not pose a problem in pf water + Tween. Therefore, we propose to keep using pf water + Tween as described in the original extraction protocols.

For glucans, we found that NaOH is not suitable as extraction solution, although Douwes *et al.* (Douwes *et al.*, 1996) found in NaOH extracts of plant material similar results as in the heated PBS–Tween extracts. Foto *et al.* (Foto *et al.*, 2004) measured glucans in outdoor air samples and Thorn *et al.* (1998) in indoor air samples both extracted in NaOH and analyzed in the modified LAL assay for glucans. This may indicate that the NaOH-soluble glucans measured by the modified LAL assay differ from the—only heat-soluble—glucans measured in our EIA. Alternatively, NaOH extraction may only produce measurable yields at higher concentrations, in which case the low glucan concentrations in our samples may have influenced our results.

We compared two glucan extraction procedures—directly from the cloth or sequentially after endotoxin extraction. Slightly higher glucan levels were found in sequentially extracted samples, but this difference was not significant and correlation between both procedures was strong. Therefore, both extraction procedures can be used for glucan extraction, but sequential extraction ensures a more efficient sample use.

We compared endotoxin and glucan yields after a sampling period of 4 weeks as opposed to the previously reported 2-week periods (Noss *et al.*, 2008, 2009). Levels of samples from both sampling periods correlated strongly for both endotoxin and glucan measurements. However, GMs were similar for endotoxin and significantly but only 51% higher for glucans after 4 weeks sampling. It is unclear, why we did not measure twice as high levels after a doubled sampling time. One explanation could be saturation of the cloths after longer sampling time. We did not find such saturation for cloths in the EDC validation study, where three times higher endotoxin levels were measured in farm than in urban homes and even 100 times higher endotoxin levels in stables—with visible dust on the cloths—than in the farm homes (Noss *et al.*, 2008) and this makes saturation highly unlikely. Resuspension of particulates as a result of air movement could be another possible explanation. None of these explanations is however completely satisfactory, and this non-linear relationship between sampling time and measured concentrations may be a typical feature of the EDC's passive sedimentation sampling technique. The most important conclusion for practical applications is that EDC exposure assessment studies should use uniform sampling times throughout to prevent unexplainable variations between measurements.

Other passive airborne dust sampling methods have previously been proposed, such as Petri dish sampling (Karlsson *et al.*, 2002a,b; Renström, 2002) or the dustfall collector (Würtz *et al.*, 2005; Hyvärinen *et al.*, 2006). The Petri dish sampling method has up to now only been applied for Fel d 1 measurement and the dustfall collector for culturable fungi and β-(1,3)-glucan. Interestingly, no studies have been reported for comparing the extraction or storage procedures for these or other house dust sampling methods—like floor or mattress dust sampling—in spite of the many studies on endotoxin measurements in various occupational environments. Applicable adaptations from protocols used previously do not necessarily mean that an optimal and accurate procedure is used. Further research in that area and especially for other components of dust apart from endotoxin is necessary in order to make accurate and precise estimations of such exposures.

## CONCLUSION

In conclusion, EDC samples from low-exposure environments can be stored for short term (up to 5 weeks) at RT, but for longer periods storage at −20°C is recommended. Endotoxin extraction should preferably be carried out in pf water + Tween. The sequential extraction for glucans grants more efficient sample use and is thus preferred. Doubling sampling time did result in unexpected similar endotoxin and only 50% higher glucan levels; therefore, uniform sampling times should be applied throughout each study.

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## REFERENCES

Bogdanovic J, Wouters IM, Sander I *et al*. (2006) Airborne exposure to wheat allergens: optimised elution for airborne dust samples. J Environ Monit; 8: 1043–8.

Douwes J, Doekes G, Montijn R *et al*. (1996) Measurement of beta(1—>3)-glucans in occupational and home environments with an inhibition enzyme immunoassay. Appl Environ Microbiol; 62: 3176–82.

Douwes J, Versloot P, Hollander A *et al*. (1995) Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. Appl Environ Microbiol; 61: 1763–9.

Foto M, Plett J, Berghout J *et al*. (2004) Modification of the Limulus amebocyte lysate assay for the analysis of glucan in indoor environments. Anal Bioanal Chem; 379: 156–62.

Hyvärinen A, Roponen M, Tiittanen P *et al*. (2006) Dust sampling methods for endotoxin—an essential, but underestimated issue. Indoor Air; 16: 20–7.

Karlsson AS, Hedren M, Almqvist C *et al*. (2002a) Evaluation of Petri dish sampling for assessment of cat allergen in airborne dust. Allergy; 57: 164–8.

Karlsson AS, Renström A, Hedren M *et al*. (2002b) Comparison of four allergen-sampling methods in conventional and allergy prevention classrooms. Clin Exp Allergy; 32: 1776–81.

Liebers V, Raulf-Heimsoth M, Linsel G *et al*. (2007) Evaluation of quantification methods of occupational endotoxin exposure. J Toxicol Environ Health A; 70: 1798–805.

Morgenstern V, Bischof W, Koch A *et al*. (2006) Measurements of endotoxin on ambient loaded PM filters after long-term storage. Sci Total Environ; 370: 574–9.

Normand AC, Vacheyrou M, Sudre B *et al*. (2009) Assessment of dust sampling methods for the study of cultivable-microorganism exposure in stables. Appl Environ Microbiol; 75: 7617–23.

Noss I, Wouters IM, Bezemer G *et al*. (2010) Beta-(1,3)-glucan exposure assessment by passive airborne dust sampling and new sensitive immunoassays. Appl Environ Microbiol; 76: 1158–67.

Noss I, Wouters IM, Visser M *et al*. (2008) Evaluation of a low cost electrostatic dustfall collector for indoor air endotoxin exposure assessment. Appl Environ Microbiol; 74: 5621–7.

Olenchock SA, Lewis DM, Mull JC. (1989) Effects of different extraction protocols on endotoxin analyses of airborne grain dusts. Scand J Work Environ Health; 15: 430–5.

Renström A. (2002) Exposure to airborne allergens: a review of sampling methods. J Environ Monit; 4: 619–22.

Reynolds SJ, Milton DK, Heederik D *et al*. (2005) Interlaboratory evaluation of endotoxin analyses in agricultural dusts—comparison of LAL assay and mass spectrometry. J Environ Monit; 7: 1371–7.

Reynolds SJ, Thorne PS, Donham KJ *et al*. (2002) Comparison of endotoxin assays using agricultural dusts. AIHA J (Fairfax, Va); 63: 430–8.

Samadi S, Heederik DJJ, Krop EJM *et al*. (2010) Allergen and endotoxin exposure in a companion animal. Occup Environ Med.

Schram-Bijkerk D, Doekes G, Boeve M *et al*. (2006) Exposure to microbial components and allergens in population studies: a comparison of two house dust collection methods applied by participants and fieldworkers. Indoor Air; 16: 414–25.

Schram-Bijkerk D, Doekes G, Douwes J *et al*. (2005) Bacterial and fungal agents in house dust and wheeze in children: the PARSIFAL study. Clin Exp Allergy; 35: 1272–8.

Spaan S, Doekes G, Heederik D *et al*. (2008) Effect of extraction and assay media on analysis of airborne endotoxin. Appl Environ Microbiol; 74: 3804–11.

Spaan S, Heederik DJ, Thorne PS *et al*. (2007) Optimization of airborne endotoxin exposure assessment: effects of filter type, transport conditions, extraction solutions, and storage of samples and extracts. Appl Environ Microbiol; 73: 6134–43.

Stone BA, Clarke AE. (1992) Chemistry and biology of (1->3)-ß-glucans.Victoria, Australia: La Trobe University Press.

Thorn J, Rylander R. (1998) Airways inflammation and glucan in a rowhouse area. Am J Respir Crit Care Med; 157: 1798–803.

Würtz H, Sigsgaard T, Valbjorn O *et al*. (2005) The dustfall collector—a simple passive tool for long-term collection of airborne dust: a project under the Danish Mould in Buildings program (DAMIB). Indoor Air; 15 (Suppl 9): 33–40.