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Surface Sampling for Endotoxin Assessment using Electrostatic Wiping Cloths

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Objectives: Much of the cost of exposure assessment for studies of residential cohorts is in scheduling and travel time for field staff. One way to reduce costs is to simplify methods such that subjects can sample their own residence. Analysis of settled dust is being widely used for assessment of exposures to allergens, lead and pesticides and can also be used for endotoxins. While vacuum sampling is the most common surface sampling method, wipe sampling has the advantage that it can be readily performed by the resident when convenient and samples can then be mailed to researchers. Thus, we evaluated the feasibility of wipe sampling for endotoxin environmental assessment using electrostatic wipes with or without the use of disposable examination gloves.

Methods: Multiple lots of six types of commercial wipes and eight types of gloves were extracted and analyzed for endotoxin content using the kinetic chromogenic *Limulus* amebocyte lysate assay. Wipes were compared across brands, between lots, within lots, between pairs depending on proximity to cardboard packaging, and in wipe tests with or without gloves. Collected dust samples of known concentration were also tested in spiking assays for endotoxin recovery.

Results: The most striking finding was the high variability of endotoxin contamination of both wipes and gloves across brands and between various lots. The content of endotoxin in unused gloves ranged from <1.5 to 5810 endotoxin units (EU). The range for unused wipes was 3.6–87.8 EU. Surfaces of equal loading and area were sampled using three types of cloths that had low initial endotoxin contamination. The cloths were very good at collecting dust and endotoxin could be assayed from aqueous extracts of the wipes. Samples collected using cloths with bare washed hands yielded higher endotoxin loading per mass of collected dust versus samples collected wearing endotoxin-free gloves. This demonstrated additional endotoxin loading from the subject's hand.

Conclusion: This study shows that wipe sampling while wearing medical gloves can be an effective method for collecting and assessing endotoxin on surfaces, so long as each lot of wipes and gloves have been tested and determined to be low in endotoxin.

Keywords: asthma; dust sampling; endotoxin; exposure assessment methodology; indoor environment

INTRODUCTION

Gram-negative bacteria are found as normal microflora of soil, water and living organisms, including humans and their pets. Endotoxin is a major cell wall component of these bacteria and is ubiquitous in the outdoor (Heinrich *et al*., 2003; Mueller-Anneling *et al*., 2004) and indoor environments (Michel *et al*., 1996; Wouters *et al*., 2000; Park *et al*., 2001; Braun-Fahrlënder *et al*., 2002; Thorne *et al*., 2003). Clinical reactions to environmental endotoxin exposure include airway inflammation, toxic pneumonitis, mucous membrane irritation and exacerbation of asthma (Clapp *et al*., 1994, Milton *et al*., 1995; Schwartz *et al*., 1995; Jagielo *et al*., 1996; Michel *et al*., 1997; Kline *et al*., 1999; Thorne and Heederik, 1999). A number of epidemiologic studies have reported associations between endotoxin exposure and respiratory symptoms or pulmonary function

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decline (reviewed in Douwes *et al*., 2003). Several large studies have evaluated the relationship between endotoxin exposure and childhood asthma. These studies have shown that early life exposure to endotoxin may have a protective effect for the development of allergy (Ernst and Cormier, 2000, Gehring *et al*., 2001; Klintberg *et al*., 2001; Braun-Fahrlënder *et al*., 2002) but may exacerbate asthma symptoms and wheezing and may lead to increased use of medications for asthma (Litonjua *et al*., 2002; Bolte *et al*., 2003). In these studies, endotoxin was sampled by vacuuming dust from floors, carpets, upholstery and bedding. This method of sampling required field workers to schedule resident-convenient appointments and to visit each household. This approach required a significant effort to contact and schedule residential visits and incurred substantial staff travel time to visit study homes. In some studies, multiple visits to each residence were required. Therefore, we have proposed an endotoxin sampling protocol that can be performed by the resident of the household at their convenience. This was motivated by our earlier experiments using electrostatically charged cloths for allergen sampling. For this method, pre-weighed electrostatic cloths are supplied and used to wipe dust from a measured area of a defined surface such as a countertop, refrigerator, dresser or bookcase. The wipes are then replaced into the clean plastic bag in which they were supplied and sent back to the laboratory for post-weighing and endotoxin analysis. The amount of dust collected and the endotoxin content of the dust serve as exposure metrics.

The aim of this study was to evaluate the efficacy of using electrostatic wipes for sampling of surfaces in order to quantify home endotoxin exposure with sampling performed by the resident. We were concerned that the electrostatic wipes used might contain endotoxin, or that their extracts would interfere with the *Limulus* assay, or that the untrained person performing the sampling (the actual resident) might contaminate the wipe with endotoxin or with bacteria. This study sought to determine the levels of endotoxin in unused wipes and gloves and evaluate endotoxin recovery from different types of cloths used in the sampling process in order to establish the feasibility of the sampling approach.

MATERIALS AND METHODS

Experimental design

Multiple lots of six types of commercially available electrostatic wipes and eight types of gloves were extracted and analyzed for endotoxin content. Medical gloves were compared between types and lots. In addition, three types of gloves were tested to determine if there was any effect of glove proximity to cardboard packaging material on endotoxin level. Further, samples of wipes were compared across brands and in wipe tests with or without medical gloves.

Endotoxin assay

All glassware was rendered pyrogen free by heating overnight at 200°C prior to use. All plastic labware was tested and verified as pyrogen free. For each sample tested, endotoxin was extracted from the entire electrostatic wipe by elution into 50 ml pyrogen-free water (BioWhittaker, Inc., Walkersville, MD) plus 0.05% Tween-20 (Sigma, Inc., St. Louis, MO) and from each entire medical glove by elution into 30 ml pyrogen-free water plus 0.05% Tween-20 in 250 ml conical pyrogen-free centrifuge tubes (Corning, Inc., Corning, NY). Wipes and gloves were shaken for 60 min, centrifuged for 20 min at 600 *g*, after which eluates were assayed immediately without freezing.

Endotoxin concentration was determined using the kinetic chromogenic *Limulus* amebocyte lysate (LAL) assay (Kinetic-QCL; BioWhittaker, Inc.), as described previously (Thorne, 2000). All reagents used for the endotoxin analysis were from the same lots. For each assay, a 12-point standard curve was generated over the concentration range 0.049–100 EU ml^{-1} and referenced to standard endotoxin (*E. coli* E50-643; BioWhittaker, Inc.). Endotoxin standards and 4-fold serial dilutions of wipe or glove extract were assayed in pyrogen-free microtiter plates (Costar no.3596; Corning, Inc., Corning, NY) in a microplate reader (SpectraMax 384 Plus, Molecular Devices, Sunnyvale, CA) for 90 min at 37°C. Spectrophotometric measurements at 405 nm were taken at 30 sec intervals. Data were analyzed using SoftMaxPro software (Molecular Devices). Sample concentrations were computed from a 4-parameter fit of the maximum reaction rate values for the standards. The minimum acceptable r^2 value of the standard curve was 0.996.

Surface sampling in homes

Three home owners received sampling kits consisting of Brand E vinyl medical gloves packaged in pyrogen free plastic bags and two wipes drawn from the inner layers of the original carton and also packaged in plastic bags. A countertop surface in each of the three homes was sampled by the resident. Each resident tested a different brand of wipe following a written protocol in which a large flat surface was marked as shown in Fig. 1 and sampled first without wearing medical gloves (white squares) and then with gloves (hatched squares). The wipes were then folded to put the sampled portion to the inside, placed in separate sealed plastic bags and returned to the laboratory for analysis. Wipes were weighed before and after sampling in a temperature and humidity controlled weighing room. Wipes were allowed to stabilize to room conditions and weighed

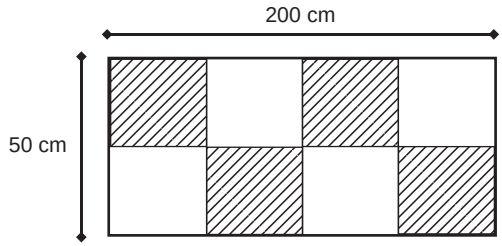

Fig. 1. Scheme for countertop wipe samples collected in three homes. Areas shown in white were sampled with ungloved but washed hands, and hatched areas were sampled while wearing disposable gloves.

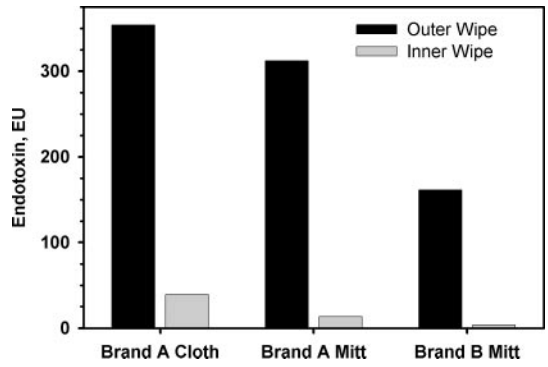

Fig. 2. Analysis of endotoxin levels from two wipes from each package shows that for these three brands, the wipes in direct contact with packaging material had more than nine times the amount of endotoxin than one taken from the middle of the package. All of these cloths were packaged in cardboard boxes without liners.

to the nearest 1 mg using an analytical balance (Mettler-Toledo, Inc., Columbus, OH).

Endotoxin recovery study

Spiking assays were performed in which three types of wipes were spiked by sprinkling sieved, vacuumed, house dust of defined endotoxin content (367.7 EU mg^{-1}) onto them. They were then extracted and assayed for endotoxin and recovery was calculated as the ratio percentage of the applied endotoxin to the assayed endotoxin after adjusting for the endotoxin content of the wipe as determined from measurement of the adjacent wipe in the package.

RESULTS

Multiple conditions must be met in order to establish endotoxin sampling using electrostatic wipes by untrained personnel as being a viable sampling approach. The sampling wipes must have a low content of endotoxin prior to sampling. The collected endotoxin must be extractable into an assayable solution that does not interfere with or enhance the LAL assay. The collection protocol must be simplified, standardized and easy to follow. The person doing the sampling must be able to collect the sample without contaminating it with other sources of endotoxin (such as might be present on hands or other handled objects in the home). To address these conditions we first had to determine that wipes with low endotoxin content were available. Three types of wipes (Brand A cloth and mitt and Brand B mitt) were first analyzed to examine the effect of the wipe packaging material (cardboard). For each brand of electrostatic wipe, two wipes were selected—one from next to the cardboard packaging (outer wipe) and the other from the middle of the package (inner wipe). In every case, the wipe next to the cardboard packaging had a much higher endotoxin level than the wipe from the middle of the package (Fig. 2). Thus, all subsequent experiments were performed using wipes and medical gloves from the middle of the packaging.

Table 1. Electrostatic wipes and medical gloves studies

Brand code	Description	Number of lots tested
Electrostatic wipes		
A	Cloth	2
A	Thick Cloth	2
A	Mitt	2
B	Cloth Scent C	2
B	Cloth Scent B	1
B	Mitt	2
Medical exam gloves		
C	Nitrile powder-free	2
D	Nitrile powder-free	1
E	Vinyl powder-free	2
F	Latex powder-free	2
G	Latex powder-free[a]	2
G	Latex sterile pre-powdered	2
H	Latex powder-free	1
I	Latex powder-free	1
J	PVC powder-free	1

[a]Glove extract inhibited the LAL assay

We next tested different lots of a variety of types of electrostatic cloths or mitts from the two leading manufacturers straight out of the package. The description of the wipes tested is provided in Table 1 and the results are shown in Fig. 3. The range of endotoxin content in the six wipe types was 3.6–87.8 EU. There was a sizable difference between wipe brands, with Brand A mitts having the lowest endotoxin levels. In addition, a difference between lot numbers of the same brand was observed in all five types of wipes for which two lots were available. The difference in endotoxin level between different lots of the Brand B mitts

was more than an order of magnitude; no other brand varied that much.

We suspected that it would be necessary to wear a medical-type disposable glove during sampling to prevent contamination of the wipe with bacteria from the hand. Thus, we tested eight types of medical gloves straight out of the packages (see Table 1 and Fig. 4). Two different lots were available for four of these and most were sampled in duplicate by drawing two non-adjacent gloves from the same package. Endotoxin content of the gloves ranged from below detection (<1.5 EU) to 5810 EU. The Brand E vinyl gloves and the Brand F powder-free latex gloves had the lowest endotoxin content, while the Brand C nitrile gloves had the highest endotoxin content. One lot of the Brand E vinyl gloves had an endotoxin content below detection (<1 EU) while the other lot yielded a mean of 17.4 EU. Although there was a difference in endotoxin between lot numbers of the same brand in all four types of gloves, the difference was smallest (20%) between different lots of the highly contaminated Brand C nitrile gloves. Brand G powder-free latex gloves inhibited the LAL assay and a determination was not possible.

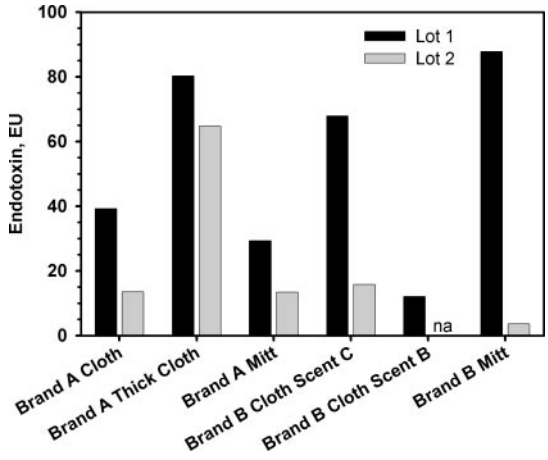

Fig. 3. Six types of electrostatic cloths taken from the middle of the packages were assayed for endotoxin content and five were tested in two lots. The lot with lower endotoxin was designated Lot 2. The lot-to-lot difference ranged from a 24% to nearly 10-fold.

We next addressed whether gloves should be worn during in-home sample collection to avoid contamination from the hand of the person collecting the sample (Fig. 5). For all three types of wipes tested with and without gloves, endotoxin levels were higher in the samples collected without wearing gloves, suggesting contamination of the wipes from the hands of the person performing the sampling. Sampling with Brand E vinyl gloves and Brand A mitts contributed a maximum of 30 EU to the samples, whereas the total endotoxin content of the Brand A mitt samples exceeded 300 000 EU.

We next evaluated recovery of endotoxin from three types of wipes (Brand A cloth, Brand A mitt and Brand B mitt) onto which we spiked varying amounts of house dust with known endotoxin content. As shown in Fig. 6, recovery varied from 37 to 96% with Brand B mitt demonstrating the highest recovery rate. The amount of endotoxin in each of

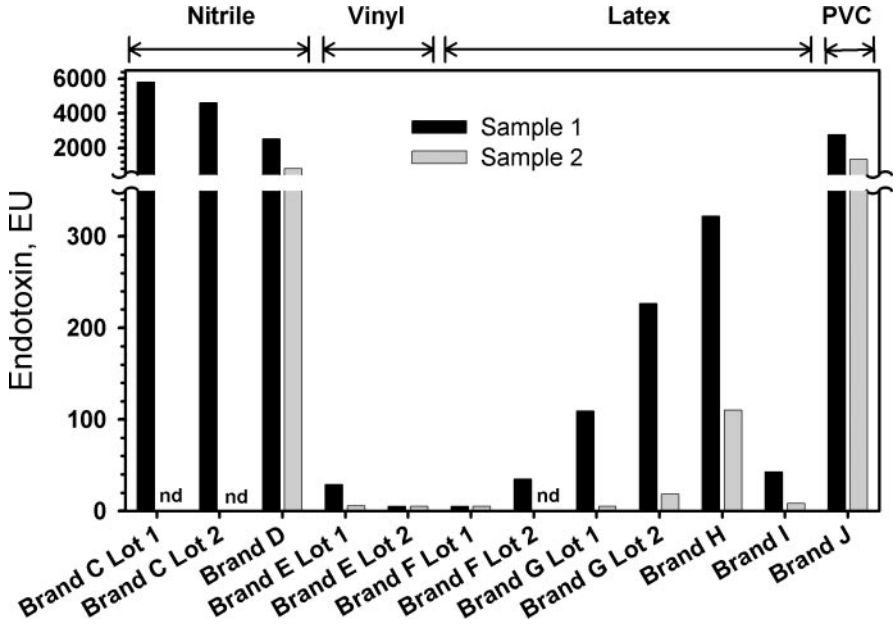

Fig. 4. Eight types of medical gloves were assayed in duplicate for endotoxin content with four tested in two lots. An additional glove type (not shown) was tested but interfered with the assay (Brand G Latex powder free). The brand and type designations are provided in Table 1. The lot with lower endotoxin was designated Sample 2.

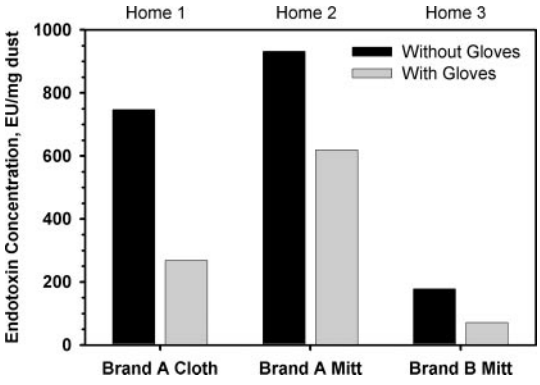

Fig. 5. Three types of low endotoxin wipes were selected to collect a wipe sample without gloves and with gloves (Brand E Vinyl Lot 2). In all cases the endotoxin level was higher in the sample collected without gloves, indicating contamination from the hands of the person sampling.

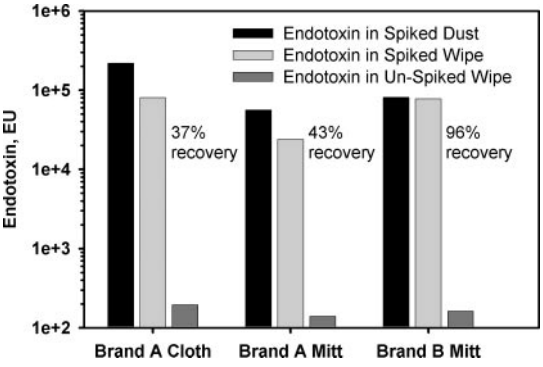

Fig. 6. Electrostatic cloths were spiked with 595, 152 or 220 mg dust containing 367 EU mg^{-1} and were extracted and assayed for endotoxin. Recovery of endotoxin ranged from 37 to 96%.

the unspiked wipes was small relative to the amount sampled (Fig. 6).

DISCUSSION

Realization of the importance of endotoxin in the pathogenesis of asthma and other respiratory diseases has created a need for effective and economical exposure assessment strategies. Current methods employed in homes and office buildings include the deployment of trained personnel to collect vacuum samples of settled dust in study locations or air samples over extended periods (e.g. 24 h). Neither of these methods facilitates enlistment of the untrained resident or office worker to perform endotoxin sampling. This study was undertaken to explore a method that removes the need to deploy trained field personnel to each and every sampling location, instead relying on the on-site resident or office worker to collect the needed sample.

Apparently, no prior studies have evaluated wipe sampling for endotoxin measurements. We found that electrostatic wipes were effective for sampling dust from smooth surfaces and that endotoxin could be analyzed from most brands and types of wipes. However, marked differences in levels of endotoxin contamination eliminated several types from consideration. Brand A cloths, Brand A mitts and Brand B mitts met the criteria of sampling well, being assayable and having one or more lots with low endotoxin.

In addition to testing wipes, we also evaluated the endotoxin content of disposable examination gloves. Williams and Halsey (1997) reported endotoxin contamination of 19 types of latex gloves ranging from 6.8 to 213 000 EU (0.9–28 400 EU g^{-1} glove). The upper end of their range far exceeded the most contaminated glove we tested. However, their analytical protocol differed from our approach. Williams and Halsey cut gloves into 0.5 cm^2 pieces and extracted 0.4 g of these pieces in pyrogen-free water, whereas we cut and eluted the entire glove into 30 ml of pyrogen-free water with Tween-20 and assayed the eluate. If the distribution of endotoxin on the gloves were uneven, they could have observed an extreme value due to sampling a smaller proportion of each glove, although it seems unlikely that this would account for a difference of this magnitude. Nonetheless, both studies demonstrated significant endotoxin contamination of medical gloves with a wide range between types.

We observed that most commercially available brands of wipes and gloves were packaged in cardboard boxes. Cardboard contains recycled fiber that is processed using water than can contain Gram-negative bacteria. We noted that gloves and wipes drawn from the middle of the box were lower in endotoxin than those from the outside. Two types of wipes (both Brand B Scented Cloths) were packaged in plastic wrappers, presumably to retain the added perfumes. One of these wipe types (Brand C Cloth Scent C) was not appreciably lower in endotoxin than wipes from the same manufacturer packaged in boxes. This suggests that cardboard packaging is not the only source of endotoxin contamination of glove and wipes.

Ideally, wipes should remove all endotoxin from the sampled surface but then release all endotoxin into the extraction solution for endotoxin assay. Limited spiking studies demonstrated 96% recovery for Brand B mitts even with a loading of 220 mg dust and 80 900 EU. This performance is quite acceptable.

CONCLUSION

This study demonstrates that it is feasible to collect scientifically-useful samples of endotoxin in homes using a simplified sampling protocol performed by

the residents themselves, removing the burden of field scheduling, appointments, and deployment travel for study staff. As with any sampling procedure, a very specific protocol would need to be followed and the potential for bias in sample collection would have to be considered. We observed that commercially available electrostatic wipes and medical examination gloves contain highly variable amounts of endotoxin between brands, types, and lots. Several brands and types of wipes and gloves were identified that were low in endotoxin contamination and could effectively be used for dust collection. It is clear that each lot of wipe and glove must be tested for endotoxin content prior to being accepted for use in sampling. The potential for endotoxin contamination by contact of the sampling wipes with the commercial packaging was also demonstrated, leading to acknowledgement of the importance of using wipes and gloves from the middle of the package. Sampling of settled dust in homes demonstrated that disposable gloves are useful to minimize hand-borne contamination of the collected wipe sample. The background contamination is low enough and the efficiency of recovery high enough to utilize commercial wipes and examination gloves for endotoxin sampling by residents in endotoxin exposure assessment.

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