Development and validation of the TefTex electrostatic wipe for indoor dust sampling

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SUMMARY
The aim of this study was to develop and validate a cost-effective method for the collection of indoor dust samples for characterization of endotoxin and ß-(1,3)-D-glucan using TefTex electrostatic wipes – pyrogen-free, polytetrafluoroethylene fibre sampling cloths. Trained research technicians visited 39 homes in Vancouver, Canada in 2009–2010 and collected two wipe samples from door tops and door frames in a bedroom and livingroom.Vacuum-collected dust samples were also obtained from flooring. Glucan and endotoxin were measured by LAL, using previously described methods. The combined endotoxin levels found on the 2 wipes were correlated with endotoxin content of vacuum collected dust ($r = 0.262$, $p = 0.021$). Similarly, combined glucan results for the wipes were correlated well with vacuum collected dust samples ($r = 0.326$, $p = 0.004$). These results suggest that TefTex wipes may provide a meaningful point-in-time means of ranking endotoxin and glucan content of dust in the home environment.

KEYWORDS
endotoxin, glucan, dust wipe, environmental sampling, electrostatic collector

1 INTRODUCTION
House dust is a matrix of organic, inorganic and composite particles originating from a wide diversity of indoor and sources and processes. Particularly in infants, exposure to dust-borne particles may occur through ingestion. As well, disturbance of settled dust by human activities or household pets results in the resuspension of particles and exposure through inhalation. Certain microbial components of dust, such as endotoxins and ß-(1,3)-D-glucan, have been associated with allergy and asthma, paradoxically showing both protective and elicitory effects (Douwes et al. 2006; Rylander 2004; Michel et al. 1991; Thorn et al. 1998).

The measurement of chemical and biological contaminants in fine dust has been used widely for the assessment of environmental exposures in population health studies. The gold-standard method for dust collection involves the use of a specially-outfitted vacuum collector operated by a trained technician (Braun-Fahrlënder et al. 2002; Ernest and Cormier 2000; Gehring et al. 2001; Klintberg et al. 2001) and analyzed for materials, typically endotoxin, ß-(1,3)-D-glucan and allergens. While effective, vacuum collection is costly and time consuming.

The aim of this study was to find a cost-effective wipe-based dust collection method that yielded measurement data comparable to vacuum-collected dust samples for the purposes of ranking indoor environmental exposures to biological contaminants. We selected a twill fabric composed of polytetrafluoroethylene (Teflon®) fiber, which remains thermostable at 250°C,

making it suitable for heat depyrogenation. Teflon is highly electronegative, promoting the electrostatic adhesion of dust to its surface. It is also very low friction and will not mar delicate surfaces, yet the material is very durable and will not tear or fray along rough surfaces.

2 MATERIALS/METHODS

Field samples of indoor dust were acquired by trained research technicians in 39 homes in Vancouver, British Columbia. Indoor dust samples were collected using the protocol implemented by the pilot phase of the Canadian Healthy Infant Longitudinal Development (CHILD) study.

Vacuum Sampling

Briefly, vacuum samples were collected using a specially designed, depyrogenated, aluminum collector for the CHILD study that holds 2 nylon thimbles (DUSTREAM™ filter DU-FL-2, Indoor Biotechnologies, Charlottesville, VA). The first sample collected consisted of a composite of the mattress and floor dust from a bedroom and the second sample was collected from the floor of the living room. A standardized floor area was sampled (2 m^2) and if insufficient sample obtained, a larger area was sampled. Research technicians visually observed the thimbles after vacuuming 2 m^2; if the thimbles were less than half-full the technician continued vacuuming in a new area of that room until thimbles were roughly half-full. The exact vacuum area size for all samples was recorded. Samples were size-fractionated using a depyrogenated 100 Tyler Mesh sieve (~150 µm), and the fine fraction was weighed, transferred to a depyrogenated borosilicate glass vial with a Teflon-lined screw cap (VWR 1 dram glass vial, West Chester, PA) and stored at –80 °C until analysis.

Wipe Sampling

TefTex wipes (5 × 5 cm squares) were depyrogenated by baking in aluminum foil at 250 °C for 3 hr, and then sealed in plastic bags until use. Wipe samples were collected in the same 2 rooms where the vacuum samples were taken. Wearing nitrile gloves, the technician collected dust from the top, horizontal surface of an interior door and the door frame. Wiping proceeded back and forth along the length of the surface, and was repeated multiple times using both sides of the wipe to get maximum retrieval. The total collection area from both surfaces represented a surface area of approximately 350–375 cm^2. Two wipe samples were collected per room. Wipes were placed back in the original foil and plastic bag and stored at room temperature until analysis. The combined endotoxin and/ or ß-(1,3)-D-glucan result for the 2 wipes used in each room was used for statistical analyses.

Extraction

Whole TefTex wipes were placed in 15 mL of extraction buffer consisting of 50 mM potassium phosphate buffer (pH 7.5) and 0.01 % triethylamine (Milton et al. 2001). Sieved, vacuum collected dust was extracted by placing 10 mg aliquots in 5 mL extraction buffer. All samples were vortex mixed for 1 min at 3000 rpm (Vortex Genie2, Scientific Industries, Bohemia, New York, USA), shaken for 20 min at 300 rpm on an orbital shaker (MaxQ 2000, Thermo Scientific, Waltham, Massachusetts, USA), then sonicated for 30 min at 26 °C (VWR Model 150HT, West Chester, Pennsylvania, USA). After 15 min, the samples were removed and vortex mixed for 1 min. The extraction was divided using a pipette to transfer 2.5 mL of the fine dust solution in to 2 borosilicate glass test tubes or 7.5 mL of the wipe eluent to 2 borosilicate glass test tubes.

Analysis

One portion of the extract was analysed for endotoxin with the Pyrochrome endpoint assay kit (Associates of Cape Cod, East Falmouth, Massachusetts, USA) (ACC). This portion was sonicated for 30 min at 26 °C, and then centrifuged at 2,000 rpm at 26 °C (Allegra X-15R Centrifuge, Beckman Coulter, Brea, California, USA). The sample was diluted using LAL water. Using a micropipette with sterile, pyrogen-free tips, duplicate 50 µL aliquots of the diluted sample were transferred to a 96-well microplate (multiple well plate 96-well, round bottom, sterile, Sarstedt, Newton, NC). 50 µL of pyrochrome reagent was added to all samples (ACC) using a repeater pipette and sterile, pyrogen-free tips. Plates were incubated at 37 °C +/– 1 °C in a heating block (Inca Personal Plate Incubator, Mikura Technology Ltd., West Sussex, UK). The incubation time to completion varied according to the Certificate of Analysis received in the kit. After incubation, the reaction was stopped with 25 µL of 50 % acetic acid to all reaction wells. Absorbancies were read by spectrophotometry at 405 nm (EL312E Bio-Tek Instruments, Inc., Winooski, Vermont, USA), and endotoxin content in each well was determined by interpolation from a standard curve. Negative controls consisting of LAL reagent water alone, and positive product controls (i.e. , spiked samples) were included in each plate.

The second portion of the extract was further extracted to analyse for ß-(1,3)-D-glucan with the Diazo Endpoint Assay (ACC). This portion of the dust extract was added to 0.5 mL of 1.8 N NaOH to give a final concentration of 0.3 N. The portion of the wipe extract was added to 0.5 mL of 4.8 N NaOH to give a final concentration of 0.3 N. The sample was shaken on ice for 30 min (MaxQ 2000, Thermo Scientific, Waltham, Massachusetts, USA), and then centrifuged for 10 min at 2000 rpm at 4 °C (Allegra X-15R). The sample was then diluted with LAL water. Duplicate 50 µL aliquots of extracted sample or diluted sample were transferred to a 96-well microplate, along with 50 µL of Glucatell® reagent (Diazo Endpoint Assay, ACC). The plate was plate incubated at 37 °C +/– 1 °C in a heating block (Inca). The incubation time to completion varied according to the Certificate of Analysis received in the kit batch. After incubation, the reaction was stopped with the addition of 50 µL ammonium sulfonate. The colour reaction was developed by the addition of 50 µL of N-(1-Napthyl) ethylenediamine dihydrochloride (NEDA). The absorbance of the reaction read through a microplate reader (EL312E Bio-Tek) set to 540–550 nm. Glucan content in each well was determined by interpolating from a standard curve. Negative controls containing only LAL reagent water and positive product controls were included in each plate.

3 RESULTS

All wipes had detectable levels of glucan, and all but 2 wipes had detectable levels of endotoxin (these wipes were given a value of zero for analysis). In a few homes there was a large variation of levels between replicate wipe samples taken in the same room; which we attributed to some rooms being cleaner than others, thus the technicians were only able to obtain a sufficient dust sample in the first wipe. As a result, statistical analysis was performed using the combined results from both wipes to gain a better measure of the overall room. Due to the loss of one dust sample and 2 dust samples having invalid results for glucan, the correlation for endotoxin was performed with 77 samples, and the correlation for glucan was performed with 75 samples. A summary of the average levels of endotoxin and glucan are given in Table 1.

The combined endotoxin results for the wipes and the vacuum collected dust were statistically correlated ($r = 0.262$, $p = 0.021$). Multivariate regression analysis determined that surface area vacuumed, floor type, amount of total dust collected, amount of fine dust collected, or percent

fine dust collected did not significantly influence this correlation. Combined glucan results for the wipes were significantly correlated with the result of vacuum collected dust samples ($r = 0.326$, $p = 0.004$). Multivariate regression analysis showed no other variable affecting the correlation.

Table 1. Mean endotoxin and ß-(1,3)-D-glucan levels in wipe-collected and vacuum-collected fine dust

Collection Method	Mean endotoxin		Mean glucan	
	(EU/g fine dust)	(EU/wipe)	(µg/g fine dust)	(µg/wipe)
Vacuum				
Smooth floor (n= 23)	206,290		439	
Carpet and smooth floor-carpet mix (n=55)	187,853		565	
Wipe				
Highest level		1,290		1

4 DISCUSSION

Previous studies have demonstrated use of electrostatic cloths in order to find a cheaper and easy collection method. Cozen et al. (2008) used Swiffer® (Procter & Gamble, Cincinnati, OH) electrostatic dust cloths for the collection of allergens, while Thorne et al. (2005) showed its effectiveness in the collection of endotoxin. Initial tests in our laboratory using the Swiffer® material showed it to be susceptible to melting during heat depyrogenation. Noss et al. (2008) demonstrated successful depyrogenation and endotoxin collection using Zeeman electrostatic cloths (Zeeman textielSupers, Utrecht, The Netherlands); however this material has limited availability. Due to these issues with the other wipes, this study compared wipes to the gold-standard vacuum collection.

Our results suggest that TefTex wipes may be an economical means of performing accurate point-in-time ranking of endotoxin and glucan levels within the home environment. Although this study used trained research technicians, the protocol can be modified to allow study subjects to sample in their own homes. With the low cost per wipe and the lack of need for a research technician, the TefTex electrostatic wipe may be a cost-effective alternative to labour-intensive vacuum dust collection method, and may facilitate longitudinal, repeated sampling of indoor environments by occupant-administered collection.

Further testing is currently being performed to determine the use of TefTex wipes to collect settled dusts for analysis of semi-volatile materials, such as phthalates, hopanes, and engine lubricants.

5 CONCLUSIONS

Dust collection has long been used to evaluate exposure to biological and chemical contaminants in population health research. Vacuum dust collection, the gold standard method for dust collection, is costly, time consuming and requires trained field personnel. Alternative collectors, such as dust wipes, use thermolabile materials that cannot readily be sterilized or depyrogenated. The background contaminant profile of these materials is largely unknown and can vary from lot to lot. Because the TefTex wipe is composed of virgin teflon twill, it can be heat depyrogenated and sterilized. It is chemically inert and will not contribute background chemical contaminants. The development of the TefTex wipe represents a major

advancement on existing wipe collector technology and offers the possibility of sample collection by study subjects as an inexpensive way to sample at multiple time points in large population health studies.

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