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PAPER

Pre-sampling contamination of filters used in measurements of airborne (1 → 3)-β-D-glucan based on glucan-specific *Limulus* amebocyte lysate assay

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Air sampling for (1 → 3)-β-D-glucan may be a good method for assessing inhalation exposure to airborne fungi. Pre-sampling contamination of filter media used for sampling (1 → 3)-β-D-glucan may lead to substantial exposure measurement errors. Using the *Limulus* amebocyte lysate assay, we tested for pre-sampling levels of (1 → 3)-β-D-glucan on three types of filters—mixed cellulose ester (MCE) [1 brand], glass fiber (GF)[1 brand], and polycarbonate (PC)[5 brands]. Levels of (1 → 3)-β-D-glucan on MCE filters exceeded 4586.1 pg per filter. Levels on GF filters averaged 135.3 (±28.9) pg per filter (range = 94.8–160.4 pg per filter) and levels on PC filters averaged 152.4 (±236.1) pg per filter (range = non-detectable–1760.7 pg per filter). Efforts to clean MCE and GF filters were unfeasible or unsuccessful. Sonicating PC filters for two hours in ethanol, followed by a wash in pyrogen-free water, effectively eliminated measured levels of (1 → 3)-β-D-glucan on four brands of PC filters, as compared to untreated PC filters. This pretreatment process did not appear to physically damage the PC filters. Air sampling results highlighted the potentially problematic contamination of untreated PC filters. Ensuring that sampling media are free of (1 → 3)-β-D-glucan before sampling is crucial to accurately measure levels of (1 → 3)-β-D-glucan exposure, especially in environments where levels of (1 → 3)-β-D-glucan are low.

Introduction

Indoor exposure to dampness and mold is recognized as a public health hazard and a plausible cause of respiratory morbidity.[1–3] Fungal and endotoxin exposures in damp office buildings may act synergistically to produce building-related respiratory symptoms.[4] While further evidence is required to show that mold and endotoxin are causal agents for these symptoms, they are at least markers for exposures in damp indoor environments that can lead to building-related respiratory illnesses.

(1 → 3)-β-D-Glucan is a structural cell-wall component of most fungi, higher plants, and some bacteria and lower plants.[5] These polysaccharides are potent pro-inflammatory agents, which can produce non-allergic inflammation in airways.[6–8] Indoor exposures to (1 → 3)-β-D-glucan are generally measured by surface or air sampling, with *pros* and *cons* existing for each method.[9] While surface dust sampling may be useful as an indicator of long-term average exposure, air samples collected from indoor environments may provide a more direct measurement of inhalation exposure.

Mixed cellulose ester (MCE), glass fiber (GF), and polycarbonate (PC) filters have been used for collection of air samples for (1 → 3)-β-D-glucan analysis.[10–12] The *Limulus* amebocyte lysate (LAL) assay is a commercially available method to quantify (1 → 3)-β-D-glucan collected on these filters. This assay has high sensitivity (~1 pg ml^{-1}); thus, filter samples with low

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Environmental impact

(1 → 3)-β-D-Glucan is a structural cell wall component of most fungi, plants, and some bacteria. Exposures to (1 → 3)-β-D-glucan have been associated with airway inflammation and respiratory symptoms. Air sampling for (1 → 3)-β-D-glucan may provide a measurement of direct inhalational exposure in indoor environments, but pre-sampling contamination of filter media used during sampling may cause inaccurate measurements of exposure. The laboratory-based pretreatment method presented in this study ensures that polycarbonate filters used in air sampling are free of (1 → 3)-β-D-glucan and enables researchers to more accurately assess exposure to (1 → 3)-β-D-glucan in epidemiologic studies carried out in indoor environments.

amounts of (1 → 3)-β-D-glucan may be reliably analyzed.[13] Levels of (1 → 3)-β-D-glucan in the air of indoor environments with little or no fungal presence may be negligible or at the lower limit of detection.[11,14–23] Therefore, if filters already have a measurable amount of (1 → 3)-β-D-glucan prior to sampling, then the detection of low levels in ambient air by the LAL assay may not be an accurate measurement of exposure. During routine analysis of air samples for (1 → 3)-β-D-glucan, we found that both laboratory and field blanks had measurable levels of (1 → 3)-β-D-glucan. Laboratory blanks were not assembled into the cassettes and did not leave the laboratory, while field blanks were assembled into cassettes and transported to and from the field but were not used for air sampling. This finding led us to further investigate the pre-sampling contamination of three types of air sampling filters and ways to eliminate this contamination.

Methods

Selection of filters

We examined three types of filters: MCE (1 brand [Millipore, Billerica, MA]), GF (1 brand [SKC, Inc., Eighty Four, PA]), and PC (5 brands [Millipore, Billerica, MA; Sterlitech Corporation, Kent, WA; Zefon International, Ocala, FL; SKC, Inc., Eighty Four, PA; Whatman Inc., Piscataway, NJ]). For all types and brands, we tested 37 millimetre filters from a previously unopened box. Because PC filters are the most commonly used of these types for airborne (1 → 3)-β-D-glucan sampling, and because our lab has a history of using PC filters for sampling, we concentrated our efforts to develop an effective cleaning treatment on this type of filter. We extensively studied one brand of PC filter because of its history of use within our lab, but also tested four other brands of PC filters to broaden our study.

Extraction of filters

We placed MCE, GF, and PC filters in 15 ml sterile conical tubes with 3 ml 0.75 normal (N) sodium hydroxide (NaOH) (one filter per tube). Throughout all experimental procedures, filters were consistently handled with sterile tweezers using aseptic technique. Using an ultrasonic cleaner (Bransonic® Model 5510, Branson Ultrasonics Corp., Danbury CT), we extracted filters for 4 hours with 10 seconds of vortexing every 30 minutes.

Determination of (1 → 3)-β-D-glucan levels

We analyzed the extract from each filter for (1 → 3)-β-D-glucan using a kinetic chromogenic LAL assay kit (Glucatell®, Associates of Cape Cod, Inc., Falmouth, MA), which included (1 → 3)-β-D-glucan-specific reagent, reagent-grade water, and (1 → 3)-β-D-glucan (pachyman) standard. The (1 → 3)-β-D-glucan-specific reagent used in these kits is a lysate of *Limulus Polyphemus* blood cells from which factor C has been removed, thereby preventing cross-reactivity with endotoxin. After thorough vortexing, we diluted the strongly alkaline filter extracts (pH 13.3) 1 : 10 and 1 : 20 in LAL reagent water (LRW) and placed a 25 μl aliquot of each dilution in a 96-well microplate. To establish a standard curve of onset time *versus* standard concentration, we assayed, in duplicate, 25 μl aliquots of (1 → 3)-β-D-glucan (pachyman) standard, ranging from 0.96 to 256 pg ml^{-1}. To check for indications of dilution-dependent interference (enhancement or inhibition) in the sample, we spiked a 100 μl aliquot of each sample dilution with 25 μl of the highest standard, and then placed 25 μl of this solution in the 96-well microplate. After reconstituting the lysate with 3.1 ml LRW and 3.1 ml 0.2 M Tris–HCl (pH 7.4), we placed 100 μl aliquots of lysate in each well containing samples, standards, spiked samples, or negative controls with a repeater pipette. The Tris–HCl buffer neutralized the alkalinity of the sample extracts. Negative controls were 25 μl aliquots of LRW and 0.2 M Tris–HCl (pH 7.4).

We placed the uncovered microplate in a plate absorbance reader (ELx808IU™, Biotek Instruments, Inc., Winooski, VT) prewarmed to 37 °C. After shaking for 10 seconds, absorbance readings at 405 nm were taken every 20 seconds for 60 minutes, for a kinetic read. The onset time of the reaction was measured as the time required for the reaction mixture to achieve a preset baseline optical density (OD) of 0.03, as recommended by the manufacturer (Glucatell®, Associates of Cape Cod, Inc., Falmouth, MA). KC4™ data analysis software (BioTek Instruments, Inc., Winooski, VT) created a log–log plot of onset times *versus* the standard concentrations. This standard curve was used to calculate the concentrations of (1 → 3)-β-D-glucan in the samples, spike samples, and negative controls. The lower limit of quantification was calculated by multiplying the smallest standard value plus the extrapolation factor (5% of standard range for lower end of the standard curve) by the smallest dilution factor and millilitres of extract.

Experiments on pretreatment of filters to remove contamination

Mixed cellulose ester filters. Because (1 → 3)-β-D-glucan levels on MCE filters were so high (>4586.1 pg per filter) and very possibly due to their intrinsic cellulosic composition (see Discussion), we chose not to evaluate pretreatments on this type of filter.

Glass fiber filters. We baked glass fiber filters for 2.5, 4, or 8 hours at 260 °C in a gravity convection oven (Lindberg/Blue M Model G01390SA-1, Thermal Product Systems, Riverside, MI) and then extracted and assayed the filters. Attempts to pretreat GF filters with liquid agents (including water and ethanol) failed because the filters disintegrated.

Polycarbonate filters

Initial pretreatment experiments. We assessed the effectiveness of various methods to pretreat PC filters: autoclaving at 133.8 °C (2.3 bar) for 20 minutes; removing dust-attracting static from PC filters with an ionizing bar (Haug Ionization, Mississauga, Ontario, Canada); soaking filters in 70% isopropyl alcohol for 2.5 hours; soaking filters in ethanol for 3 hours; and sonicating filters in ethanol for 2 hours. We also evaluated PC-filters certified as an endotoxin-free and purchased in preassembled sampling cassettes (SKC, Inc., Eighty Four, PA) by extracting and assaying the filters as described previously. Because these initial pretreatment experiments showed that sonication of filters in ethanol was the most effective at reducing levels of (1 → 3)-β-D-glucan, we focused further efforts on this method of pretreatment.

Sonicating PC filters in ethanol. We placed five brands of PC filters in 50 ml conical tubes with 20 ml ethanol (one filter per tube). Using the ultrasonic cleaner, we sonicated the filters for two hours and then removed them aseptically from the conical tubes. We washed the filters thoroughly by individually submerging each one for 10 seconds in 500 ml pyrogen-free water (Baxter International Inc., Deerfield, IL), followed by duplicate 10 second submersions, each in a separate 250 ml volume of pyrogen-free water. We changed the water in the 500 ml wash every 12 filters; we changed the water in the 250 ml washes every six filters. We then dried the filters overnight in a biosafety cabinet. The following morning, we extracted and assayed the pretreated filters, as described previously.

Scanning electron microscopy. One untreated PC filter and one PC filter which had been sonicated in ethanol were placed on pin type aluminium scanning electron microscopy (SEM) specimen mounts (Electron Microscopy Sciences, Hatfield, PA) with carbon adhesive tabs (Electron Microscopy Sciences) for SEM analysis. Each sample was coated with a thin layer of gold/palladium utilizing a sputter coater (SPI-Module™ system, Structure Probe, Inc., West Chester, PA). Images were produced using a SEM under computer control (JSM-6400, JEOL Ltd., Tokyo, Japan) at 2000× magnification.

Field air sampling. We collected six pairs of side-by-side air samples (total of 12 samples) in three homes (three living rooms, one home office, and one family room) and one research building (one office). For this, we used an air pump (SP 280, Air Diagnostics and Engineering, Inc., Naples, ME) sampling at a flow rate of 4 l min^{-1}, with a two-piece closed cassette (Millipore, Billerica, MA) attached for up to 80 hours (identical sampling time for the two filters in each pair) with untreated 0.8 μm PC filters (Brand 1) and 0.8 μm PC filters (Brand 1 from the same box) pretreated with sonication in ethanol. Plastic pads (SKC, Inc., Eighty Four, PA) were used to support the filters in the cassettes. After sampling, we extracted and assayed the filters as described previously.

Data analysis

We computed mean and standard deviations using Microsoft Excel® software. Coefficients of variation were computed as a ratio of standard deviation to mean. Because this study was exploratory, without a formal experimental design, we refrained from conducting more extensive statistical analyses.

Results

Measured levels of (1 → 3)-β-D-glucan in untreated filters

Amounts of (1 → 3)-β-D-glucan measured on untreated MCE filters were all above the highest concentration of the standard curve (>256 pg ml^{-1}) (Table 1). The onset times for these reactions occurred sooner than the onset time of the highest concentration of the standard, and thus the concentration of the (1 → 3)-β-D-glucan in the extracts of MCE filters was not determined. The four untreated GF filters averaged 135.3 (±28.9) pg per filter [mean (±standard deviation)], ranging from 94.8–160.4 pg per filter. Overall, levels on PC filters averaged 152.4 (±236.1) pg per filter. Measured amounts of (1 → 3)-β-D-glucan on PC filters ranged from non-detectable to 1760.7 pg per filter. PC filters from Brand 1 (n = 41) had a mean of 126.3 (±86.4) pg per filter, while PC filters from Brands 2, 3, and 5 together (n = 12) had a mean of 87.4 (±98.2) pg per filter. PC

Table 1 Levels of (1 → 3)-β-D-glucan by filter type and pretreatment/condition[a,b]

					(1 → 3)-β-D-glucan/pg per filter	
Filter type	Pretreatment/condition		Brand	No. of filters	Mean	SD
Mixed cellulose ester	None		—	4	>4586.1	—
Glass fiber	None		—	4	135.3	28.9
	Baked at 500 °F	2.5 hours	—	6	143.7	163.1
		4 hours	—	2	222.8	181.3
		8 hours	—	3	278.4	126.1
Polycarbonate	None		1	41	126.3	86.4
			2, 3, 5	12	87.4	98.2
			4	4	614.1	771.8
	Autoclaved		1	3	220.6	50.9
	Static removed		1	7	205.4	114.4
	Soaked 2.5 hours in 70% isopropyl alcohol		1	2	123.7	65.8
	Soaked 3 hours in ethanol		1	3	124.0	73.2
	Sonicated 2 hours in ethanol, followed by pyrogen-free water wash		1	22	21 of 22 < LOD[c]	—
			2, 3, 5	8	8 of 8 < LOD[c]	—
			4	4	1 of 4 < LOD[d]	—
Polycarbonate (preassembled in cassette)	Certified endotoxin-free		4	2	502.2	214.0

[a] SD, standard deviation; LOD, limit of detection. [b] The (1 → 3)-β-D-glucan level on all 4 untreated MCE filters exceeded the highest concentration of the standard curve, which was 256 pg ml^{-1} by our methods. [c] 1 of 22 Brand 1 filters pretreated by sonication in ethanol had a measurable level of (1 → 3)-β-D-glucan (112.9 pg). Levels on the other 21 Brand 1 filters were all below the limit of detection, which was 2.3 pg per filter by our methods. [d] 3 of 4 Brand 4 filters pretreated by sonication in ethanol had measurable levels of (1 → 3)-β-D-glucan (102.6 ± 79.6 pg per filter) The level on the other Brand 4 filter was less than the limit of detection, which was 2.3 pg per filter by our methods.

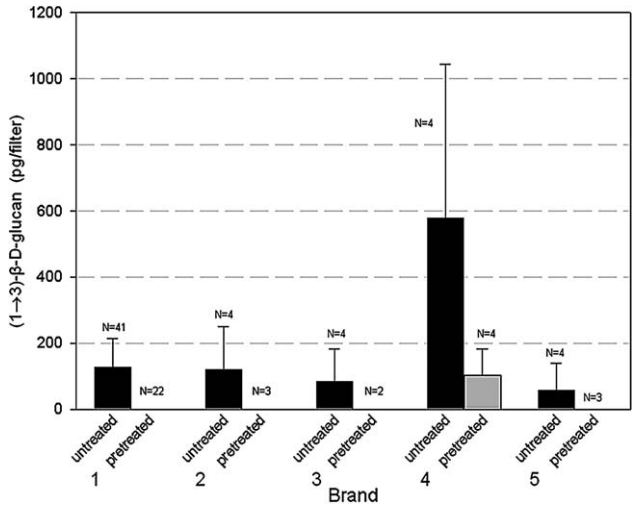

Fig. 1 Levels of (1 → 3)-β-D-glucan in five brands of polycarbonate filters, pretreated with sonication in ethanol and untreated. Error bars represent standard deviation. Except for 1 filter from Brand 1, for which (1 → 3)-β-D-glucan measured 112.9 pg per filter, levels of (1 → 3)-β-D-glucan on pretreated polycarbonate filters from Brands 1, 2, 3, and 5 were all below the lower limit of detection (2.3 pg per filter).

filters from Brand 4 ($n = 4$) averaged 614.1 (±771.8) pg per filter, indicating much higher pre-sampling contamination and variability than the other four brands (Fig. 1). Brand-specific coefficients of variation were all greater than 68 (range: 68.4–137.4) percent.

The two tested PC filters marketed as certified endotoxin-free filters in preassembled sampling cassettes had a mean level of (1 → 3)-β-D-glucan over three times higher than the mean of other untreated PC filters (excluding Brand 4) (Table 1).

Effect of pretreatment of glass fiber and polycarbonate filters

After baking GF filters for 2.5, 4, or 8 hours at 260 °C, (1 → 3)-β-D-glucan levels were still measurable, with high variability. The mean amount of (1 → 3)-β-D-glucan on all baked GF filters was 194.8 (±153.6) pg per filter, as compared to a mean of 135.3 (±28.9) pg per filter on the untreated GF filters. As baking times increased, so did the mean amount of measured (1 → 3)-β-D-glucan on baked GF filters.

Autoclaving PC filters was unsuccessful in lowering the measured level of (1 → 3)-β-D-glucan in these filters (Table 1). The mean level of (1 → 3)-β-D-glucan on autoclaved PC filters was higher than on untreated PC filters, but individual levels were less variable. Removing dust-attracting static from untreated PC filters likewise did not diminish the measured level of (1 → 3)-β-D-glucan on PC filters, as compared to the mean level of (1 → 3)-β-D-glucan on untreated filters. Soaking PC filters in 70% isopropyl alcohol or ethanol and then air-drying them in a biosafety cabinet only slightly decreased the measured level of (1 → 3)-β-D-glucan on these filters, as compared to the mean level of (1 → 3)-β-D-glucan on untreated filters.

Pretreatment of 34 PC filters by sonication in ethanol for two hours, followed by a thorough wash in pyrogen-free irrigation water and air-drying overnight in a biosafety cabinet, removed all measurable amounts (limit of detection = 2.3 pg per filter) of (1 → 3)-β-D-glucan contamination on all but four filters (Fig. 1). One of these PC filters with measurable residual (1 → 3)-β-D-glucan was from Brand 1 and had a measured level of 112.9 pg per filter. The other three PC filters with measurable residual (1 → 3)-β-D-glucan were from Brand 4 and had a mean level of 102.6 (±79.6) pg per filter.

Scanning electron microscopy of pretreated *vs.* untreated PC filters

At 2000× magnification, the SEM images indicated no apparent difference in physical appearance between the untreated PC filters and those pretreated with sonication in ethanol (Fig. 2).

Air sampling using pretreated *vs.* untreated PC filters

In four of six sampling locations, the measured level of (1 → 3)-β-D-glucan was higher on the untreated filters compared to the

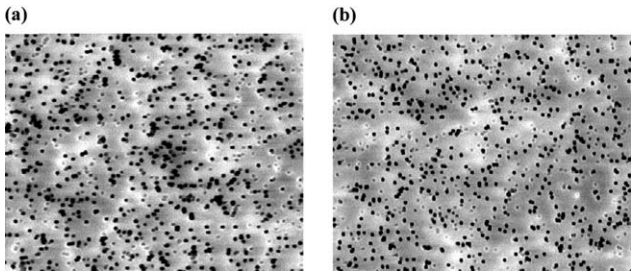

Fig. 2 Direct scanning electron microscopy (SEM) photomicrographs (2000× magnification) of polycarbonate filters, one untreated (a) and the other pretreated with sonication in ethanol (b).

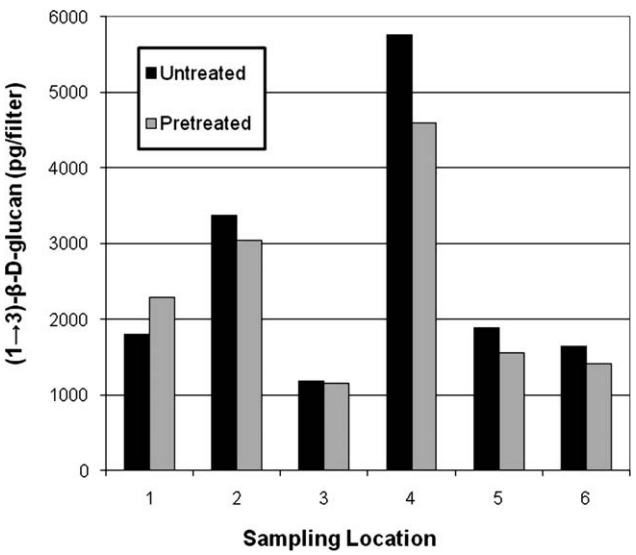

Fig. 3 Levels of (1 → 3)-β-D-glucan in six paired sets of field samples for six indoor locations. Paired sampling was done using polycarbonate filters, one pretreated with sonication in ethanol and the other untreated. Locations 1–3 were in living rooms of Homes 1 (sampling time = 61 hours), 2 (72.6 hours), and 3 (73 hours), respectively. Location 4 (80.8 hours) was in the office area of Home 2. Location 5 (47.7 hours) was in the family room of Home 3. Location 6 (55.8 hours) was in an office in a research building.

pretreated filter from the same sampling location (Fig. 3). In these four sampling locations, the mean of the differences in the concentrations of (1 → 3)-β-D-glucan between paired samples was 512.4 (±436.5) pg per filter. The difference in the treated and untreated filters ranged from 2 to 20 percent of the concentration of (1 → 3)-β-D-glucan on the untreated filters, for these four sampling locations.

Discussion

Air sampling for (1 → 3)-β-D-glucan may be a useful method for assessment of exposure to fungi, and exposure to (1 → 3)-β-D-glucan itself has been shown to be associated with health effects.[1] However, contamination of filters used in the sampling process could prevent the accurate assessment of exposure to airborne (1 → 3)-β-D-glucan, especially when low levels are assessed. Our study demonstrates that three types of filters used for sampling airborne (1 → 3)-β-D-glucan were contaminated with up to 1.76 ng per filter of (1 → 3)-β-D-glucan even before they were used for sampling. Inaccurate measurement of airborne (1 → 3)-β-D-glucan due to highly variable pre-sampling contamination of the filters would lead to non-differential misclassification of exposure in epidemiological studies, which may result in attenuation of any associations between exposure and health effects.

Multiple studies have reported low levels of airborne (1 → 3)-β-D-glucan in homes, as measured by the LAL assay.[11,14–16,18–21] Reported geometric means in many of those studies ranged between 1 and 2 ng m^{-3} and were even lower than 1 ng m^{-3} for size selective samples,[15,19,21] which implies that a significant portion of air samples may have a concentration of (1 → 3)-β-D-glucan lower than or around 1 ng m^{-3}. Concentrations of airborne microbial agents including (1 → 3)-β-D-glucan in uncontaminated office building environments are typically even lower than those in homes.[22,23] If both measured concentration and sampled air volume are low (*e.g.* <2 m^3), the relative contribution of filter contamination to the concentration of (1 → 3)-β-D-glucan may be substantial and should not be ignored. Based on the mean plus one SD of untreated blank PC filter concentrations measured in our study, and assuming a normal distribution, about 16% of PC filters might be contaminated with higher than 0.2 (Brands 1, 2, 3, and 5) to 1.4 ng (Brand 4) of (1 → 3)-β-D-glucan or glucan-like materials per filter. The large variability of (1 → 3)-β-D-glucan concentrations on untreated blank PC filters indicates that airborne (1 → 3)-β-D-glucan concentrations reported by investigators may be falsely elevated due to the variable contamination of the PC filter.

Factor G in the LAL assay may only be activated by (1 → 3)-β-D-glucan and not by other glycan structures with alternative linkages.[24] However, it is possible that other polysaccharides could interfere with the LAL assay for (1 → 3)-β-D-glucan (*e.g.* dilution-independent interference). In fact, it has been reported that polysaccharides such as xylan ((1 → 4)-β-D-glucan), mannan, and galactan activate factor G at very high concentrations,[25] though such reports may be erroneous if the observed activation was not due to these other polysaccharides but due to trace contamination with (1 → 3)-β-D-glucan.[26] Because cellulose is composed of (1 → 4)-β-D-glucan and may also be contaminated with (1 → 3)-β-D-glucan,[13,27] we suspect that the presampling contamination of the PC filters we observed might result from packaging. To prevent filters from sticking together, each PC filter is individually packaged between paper (cellulose) dividers having the same dimensions as the PC filters. When we assayed six of these paper dividers from two boxes of PC filters, the measured amount of (1 → 3)-β-D-glucan in the divider extracts that were diluted less than 2000 times exceeded the highest concentration (256 pg ml^{-1}) of the standard curve. When the divider extracts were diluted 2000 times or more, the measured (1 → 3)-β-D-glucan concentration ranged from 2.9 to 5.4 microgram per divider. This suggests that the paper dividers are a likely source of pre-sampling contamination in PC and other filters. Packaging with the paper dividers may also be responsible, at least in part, for the measured (1 → 3)-β-D-glucan contamination of GF filters and MCE filters. However, we suspect that it may have been the intrinsic cellulosic composition of MCE filers that resulted in the apparent high levels of (1 → 3)-β-D-glucan we measured in MCE filters. Therefore, we did not explore options for pre-treatment of MCE filters, nor can we recommend them for use in air sampling for (1 → 3)-β-D-glucan.

Because we found highly variable amounts of (1 → 3)-β-D-glucan in the untreated PC filters which produces random error in measurements, subtracting a mean amount found on blank filters from the amounts measured after air sampling using untreated sample filters would not be a good option to arrive at a more accurate estimate of (1 → 3)-β-D-glucan levels in the air. To accurately assess levels of (1 → 3)-β-D-glucan exposure based on air sampling using PC filters, filters that have no measurable level of (1 → 3)-β-D-glucan should be used, especially where levels of (1 → 3)-β-D-glucan are low, as they often are in indoor air sampling. Alternatively, one may collect large volume of air by increasing the sampling time or flow rate to minimize the relative contribution of the pre-sampling contamination to measured concentration; however, these options are often impractical in indoor environmental studies due to occupants' complaint of noise from samplers.

Pretreatment of PC filters with sonication in ethanol to remove background (1 → 3)-β-D-glucan contamination uses common laboratory chemicals in a simple procedure that can be easily applied to treat PC filters before their use in air sampling. With the exception of Brand 4 filters, which might be characterized as an "outlier" in comparison to the other four brands we tested in terms of measured levels of (1 → 3)-β-D-glucan contamination, we found this pretreatment method was very effective. Other pretreatment methods tested in our study were ineffective in removing (1 → 3)-β-D-glucan contamination from GF and PC filters (Table 1). While sonication in ethanol was not entirely effective in removing (1 → 3)-β-D-glucan contamination from Brand 4 filters, this pretreatment method did substantially lower measured levels of (1 → 3)-β-D-glucan even on this brand, as compared to untreated PC filters from Brand 4.

Our pretreatment method of sonicating the filters for two hours in ethanol, followed by a water wash and air dry, does not appear to damage the integrity of PC filters, as shown by SEM. Our limited field air sampling results revealed generally lower levels of measured (1 → 3)-β-D-glucan using pretreated as compared to untreated PC filters, a difference we attribute to pre-sampling contamination of untreated filters. This highlights the potential for overestimating concentrations of (1 → 3)-β-D-glucan in air if sampling is done with contaminated PC filters.

Our study was limited to MCE, GF, and PC filters from a limited number of brands and, except for Brand 1, we tested only one lot from each brand. Filter types, brands, and lots not tested might have lower or higher measured levels of (1 → 3)-β-D-glucan than what we found and report in this study. Another limitation is the low number of side-by-side air samples.

Conclusion

In this exploratory investigation we have documented the contamination of three types of sampling filters (MCE, GF, and PC) with (1 → 3)-β-D-glucan or other glucan-specific LAL-reactive materials and high variability of this contamination. These findings indicate potential for variable overestimation and misclassification of (1 → 3)-β-D-glucan exposure determined using the *Limulus* amebocyte lysate assay. Ensuring that measurable amounts of (1 → 3)-β-D-glucan in air samples stem from the environment and not from the sampling media is essential to obtain an accurate assessment of exposure to (1 → 3)-β-D-glucan for epidemiologic studies in the indoor environment. As demonstrated in this study, pretreatment of PC sampling filters by sonication in ethanol is simple and easily done in the laboratory. Until filters that are certified as (1 → 3)-β-D-glucan-free become commercially available from manufacturers, use of the pretreatment method described in this paper can help researchers to more accurately assess exposure to (1 → 3)-β-D-glucan in epidemiologic studies carried out in indoor environments.

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