Influence of sample mass on the measurement confidence of microbial cell wall materials in vacuum-collected floor dust

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SUMMARY
Technological advancements have increased the analytical sensitivity of a wide range of assays, permitting the analysis of vanishingly small concentrations of analyte. Accordingly, the minimum quantity of sample needed for analysis has become increasingly driven by the ever greater analytical sensitivity of laboratory methods with little consideration of the influence of decreasing sample mass on the representativeness of the sampled environment. Here we investigate the influence of sample mass on the measurement of endotoxin and glucan in house dust. Using a combination of empirical sampling and modeling, we demonstrate that a minimum of 150 mg of dust is needed for analysis in order to achieve a measurement precision of 20% relative standard error (RSE).

KEYWORDS
Endotoxin, glucan, population health, house dust, indoor air

1 INTRODUCTION
House dust is a particulate matrix generated by the ongoing passive deposition of airborne suspended particles on indoor surfaces, especially flooring. Accumulated dust also serves as a reservoir from which particles become resuspended by human activity, further contributing to the airborne particle burden (Ferro et al., 2004). The characterization and measurement of contaminants in dust is used widely in population health research to infer exposures.

Two chief sources of laboratory error are recognized: 1) procedural error (e.g., pipetting error, weighing error, etc.); and 2) analytical sensitivity of the method (the smallest amount of analyte accurately measured). Analytical sensitivity describes the concentration of analyte below which the signal-to-noise ratio of the method (σ/μ) fails to provide an expected level of confidence. Diminishingly small analyte concentrations are thus associated with exponentially increasing variance, as described by the so-called Horwitz function (Horwitz et al., 1980).

Here we examine a third, seldom discussed contributor to error that is unique to particulate analytes such as endotoxin and glucan: the physical scale of the analyte in relation to that of the sample matrix. As the scale of the sample approaches that of the analyte, the probability of sampling a representative and quantitatively meaningful concentration of analyte becomes exponentially more dependent on analyte homogeneity and concentration; thus, the relative standard error (RSE) (σ/μ) increases accordingly. Careful optimization of the scale of the sample (e.g., mass) is required to minimize analytical uncertainty to a pre-determined level.

2 MATERIALS/METHODS
Floor dust was collected from an occupied house in Edmonton, Alberta, Canada using a HEPA filtered vacuum cleaner (Mighty Mite, Eureka Co., Charlotte, North Carolina, USA) outfitted with a custom-designed depyrogenated aluminium tool containing two nylon mesh

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dust collection thimbles (DustStream, Indoor Biotechnologies, Charlottesville, Virginia, USA). Collection was made from a 4 m^2 area. with each swath subjected to 7 passes of the collection tool. The sample was homogenized and sieved using a depyrogenated Tyler 100 Mesh sieve (~150 μm) by vigorous mechanical shaking for 20 min, and the fine fraction transferred to a depyrogenated borosilicate glass vial with a Teflon-lined screw cap (VWR 1 dram glass vial, West Chester, Pennsylvania, USA) and stored at –80 °C until analysis.

A 10 mg aliquot of sieved fine dust was extracted for each analysis. Endotoxin was extracted in depyrogenated water (LRW) with 0.05% Tween and sonicated. Glucan was extracted with 0.5 N NaOH under sonication, neutralized with 1.0 M Tris-HCl (Foto et al., 2004). Extracts were serially diluted, reconstituted Pyrochrome LAL reagent (Associates of Cape Cod (ACC), East Falmouth, Massachuetts, USA) was added to the extract at a ratio of 2:1, incubated at 37 °C, and the OD measured at 405 nm. Standard curves of known concentrations of endotoxin (ACC) and curdlan (Supelco, Bellefonte, Pennsylvania, USA) were used to infer extract concentration. Concentrations were expressed as endotoxin units (EU) per milligram dust or nanograms glucan per milligram dust. A total of 80 replicate dust samples were analysed for endotoxin, and 80 for glucan. All analyses were performed by a laboratory proficient in the specific assays and accredited to ISO/IEC 17025:2005.

Summary statistics were computed with Microsoft Excel 2007 and Minitab 16. Using a custom-designed Visual Basic algorithm, each of the two data sets was then resampled with replacement for 1,000 replicates as a basis to generate model summary statistics for pooled aliquot masses ranging from 20 to 400 mg. Trends in the relationship between modelled aliquot mass and RSE were fitted by regression using a power model.

3 RESULTS

Approximately 4 g of fine dust was collected. Endotoxin and glucan concentrations are given in Table 1. Observed RSE for both analytes was unexpectedly high, despite that duplicate measures of the same extract showed only nominal variation (not shown). An inverse power relationship was found between RSE and modelled aliquot mass for both analytes (Figure 1).

Table 1. Summary of concentrations of endotoxin and beta-(1,3)-D-glucan content of dust

Analyte	Mean (μ)	Standard deviation (σ)	RSE (σ/μ)
Endotoxin	11,237 EU/mg	8,419	0.75
Beta-(1,3)-D-glucan	1,183 ng/mg	958	0.81

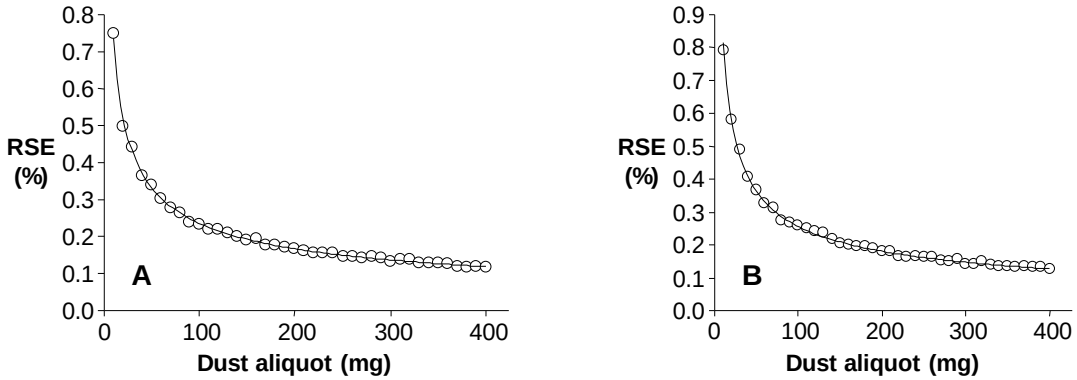

Figure 1. Relationship between dust aliquot mass and RSE of the analytes a) endotoxin (R^2=0.997) and b) beta-(1,3)-D-glucan (R^2=0.996). Each point represents the calculated RSE of 1,000 sums of resampled data, pooled for each aliquot mass-increment.

Under the conditions of our model, the relationship between RSE and aliquot mass is given by the following equation:

$$\Phi_E \approx \frac{K}{\sqrt{M}} \quad (1)$$

where Φ_E is the expected variance (% RSE), M is the mass in milligrams of extracted dust, and K is an analyte-specific constant. A K-value of 2.3 was found for endotoxin, and 2.6 for glucan.

4 DISCUSSION

Determination of the sample mass used for laboratory analysis of dust-borne contaminants tends to be driven largely by the analytical sensitivity of the method. While this approach seems intuitive, it lacks an appreciation of the influence of the sampling curve of the analyte to the overall error of the method. With most dust-borne analytes, we anticipate that the sampling curve is the largest single contributor to uncontrolled error.

Error related to sampling curve is governed both by the analyte concentration (lower analyte is associated with greater error, as described by the Horwitz function) and homogeneity of the sample. The latter is strongly influenced by the size distribution of particles in the dust matrix, and the relationship between the analyte of interest and particular categories of particles upon which the analyte is borne. Without knowledge of the size distribution of the target analyte itself, it is impossible to predict the sample size threshold that minimizes uncertainty to a pre-determined level. In order to minimize the contribution of sample mass to the overall methodological error, an empirical optimisation of sample mass is required. We fully expect that the optimal minimum aliquot size will vary, perhaps substantially, from analyte to analyte, given the nature of the size distribution of dust particles and the affiliation of certain particles with certain analytes.

Our approach of using jack-knifed resampling of pooled empirical measurements taken from uniformly sized aliquots assumed that the population we sampled was sufficiently large to include the full range of true variation. Although the original datasets of endotoxin and glucan results were log-normally distributed (data not shown), Central Limit Theorum predicts that a series of resampled sums will converge on a normal distribution, and this was consistent with our observations.

A wide range of aliquot masses have been reported in the literature for endotoxin and glucan assays, ranging from 10 mg (e.g., Tavernier et al., 2004) to 100 mg or greater (e.g., Thorne et al., 2005). Under our test conditions, we found that aliquot masses of 50–60 mg reduced error to 30%, and the use of 150 mg aliquots further reduced error to 20%. These findings are consistent with the empirical approaches taken by other groups to the optimisation of aliquot mass by analysis of replicate samples of increasing mass (Peter Thorne, personal communication).

Although we did not consider the contribution of the analytical procedures themselves to overall analytical precision (i.e., procedural error), our use of jack-knifed resampling of 10 mg samples to construct model data of incrementally larger aliquots integrated error from all sources. Procedural error becomes more pronounced at lower concentrations of analyte (Horwitz et al., 1980). Procedural error is also cumulative. Hence, we expect, for example, that the procedural contribution to overall analytical error in 10 subsamples of 10 mg would

be much greater than that present in the analysis of a single sample of 100 mg. Therefore, we anticipate that our model over-represents the contribution of the procedural error component and therefore reasonably represents a "worst-case-scenario".

5 CONCLUSIONS

In this study, we investigated the influence of sample mass on the analysis of endotoxin and glucan in house dust. Based on empirical sample data and model extrapolation, we demonstrated that a minimum of 60–70 mg of dust is required to achieve an RSE of 30% in the analysis of endotoxin and glucan, and that 150 mg is required to further reduce RSE to 20%. This finding has important implications for environmental health research, particularly cohort studies, where dust samples are collected and analysed as proxies of air exposures to endotoxin and glucan. The establishment of minimum sample sizes needed for representative exposure measures will ensure consistency across study platforms and optimise the use of valuable biological samples.

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