



Allergen Quantification by Use of Electrostatic Dust Collectors (EDCs): Influence of Deployment Time, Extraction Buffer, and Storage Conditions on the Results

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ABSTRACT

Sampling of endotoxin, beta-glucan, or allergens on electrostatic dust collectors (EDCs) is a convenient method for exposure assessment. However, especially for allergens few experiments on validation of this method concerning deployment time or storage and extraction procedure have been performed. The aim of study was to optimize the EDC procedure for sampling of allergens in indoor environments. EDCs were placed in households or day-care centers and after extraction, allergens were quantified by six immunoassays detecting mite antigens (Domestic mites DM, *Dermatophagoides pteronyssinus* Dp, *Tyrophagus putrescentiae* Tp) or the main allergens from cat (Fel d 1), dog (Can f 1) and mouse (Mus m 1). For 20 EDC holders, deployment times of cloths were varied between 7 and 28 days, 36 EDCs were used to test reproducibility, and for 28 EDCs extraction buffers were varied (with or without 0.05% Tween 20, borate, or phosphate buffer). The influence of storage of cloths at room temperature (2–629 days) or extracts at –80°C (7–639 days), and variation of extract storage temperature (–20°C and –80°C) for long time storage (1.5 years) on the outcome of allergen quantification were tested for about 150 EDCs. The allergens on EDC cloths increased proportionally with deployment time, and allergen loads on parallel sampled tissues were significantly correlated ($P < 0.0001$, Pearson of log-transformed values 0.91–0.99). Extraction without Tween reduced all results ($P < 0.0001$, –51% DM, –85% Dp, –60% Tp, –99% Fel d 1, –86% Can f 1, –52% Mus m 1), and borate buffer resulted in lower yields of Mus m 1 (–53%), DP (–45%), and Tp (–27%) than phosphate buffer. Storage of cloths at room temperature significantly decreased Can f 1 levels ($P < 0.0001$, –4.8% loss for every 30 days), whereas storage of extracts at –80°C decreased DM results ($P < 0.0001$, –1.2% loss for every 30 days). Extracts stored at –20°C gave at mean 12% higher DM results compared to extracts stored at –80°C for 1.5 years. Several mammalian allergens and also DM antigens could be quantified reproducibly on EDCs from indoor environments. Allergen levels on EDC cloths increased proportionally with deployment time in a period of 4 weeks. Allergen yields are strongly influenced by the extraction procedure; the use of detergent Tween 20 and phosphate buffer is recommended.

KEYWORDS: allergen stability; electrostatic dust collector; exposure assessment; indoor allergens; reproducibility of allergen quantification; sampling efficiency.

INTRODUCTION

Electrostatic dust collectors (EDCs) were developed by Noss *et al.* (2008) for passive sampling of airborne particles carrying endotoxins, beta-glucans, or different allergens. The EDC consists of a polypropylene sampler that contains either four or two polyester cloths providing electrostatic sampling surfaces for settled airborne dust. The advantage of this device in comparison to active sampling methods lies in the ease of use in large population studies; the samplers are inexpensive and can be sent to study participants and back to the analyzing laboratory by mail. The advantage in comparison to reservoir sampling of vacuumed dust is that the settled dust on EDCs should be more representative of the inhalable dust fraction because in contrast to EDCs, surface dust from floors or mattresses usually contains large and heavy particles that never become airborne.

Thus, several studies have used EDCs for sampling of endotoxin (Samadi *et al.*, 2010; Liebers *et al.*, 2012; Jacobs *et al.*, 2013; Jacobs *et al.*, 2014), beta-glucans (Noss and Wouters *et al.*, 2010; Krop *et al.*, 2014), micro-organisms (Normand *et al.*, 2009; Frankel *et al.*, 2012; Madsen *et al.*, 2012; Scherer *et al.*, 2014; Adams *et al.*, 2015; Normand *et al.*, 2016; Rocchi *et al.*, 2015a, b), and allergens (Samadi *et al.*, 2010; Zahradnik *et al.*, 2011a, b; Krop *et al.*, 2014; Schlünssen *et al.*, 2015). However, especially for allergens few experiments on validation of this method concerning deployment time or storage and extraction procedure have been performed. For endotoxin and beta-glucan, Noss *et al.* (2010) found no influence of sampler storage time but increased yields using Tween during extraction. Doubling the sampling duration yielded similar endotoxin and only 50% higher glucan levels. In contrast to that, Kilburg-Basnyat *et al.* (2015) observed that endotoxin loading of the EDCs doubled from 7- to 14-day deployments and increased 1.38-fold from 14 to 28 days of sampling in farm house living rooms. Furthermore, they analyzed allergen levels on these EDCs. The main allergens of cockroach (Bla g 2), dust mite (Der p 1, Der f 1, mite group 2), and rat (Rat n 1) were mostly below detection, but Fel d 1, Can f 1, and Mus m 1 could be measured by a fluorescent multiplex array kit. They considered the measurement of these allergens as reliable at longer deployment times

only. However, the allergen extraction in their protocol was performed without the addition of detergent although Tween 20 was added before centrifugation.

Considering the positive influence of detergent in extraction buffers on the amounts of various allergens (Raulf *et al.*, 2014), we used extraction buffers with or without detergent for parallel sampled cloths in EDCs and compared the outcome. Instead of quantifying single mite allergens with monoclonal antibodies, however, mite antigen assays based on polyclonal antibodies were used which were shown to have higher detection rates when airborne dust and EDCs were analyzed (Sander *et al.*, 2012; Krop *et al.*, 2014). Furthermore, main allergens of cat, dog and mouse were quantified.

The aim of study was to find the optimal procedure for quantifying allergens on EDCs in an indoor environment, including sampling, extraction, and storage of samples before analysis. EDCs were sampled in day-care centers and private homes as methodological part of a study on comparison of allergen yields at both locations. Reproducibility of sampling was tested by comparing the allergen yields of cloths that were sampled, extracted and tested in parallel. To find out the influence of sampling duration on allergen yield, the deployment times of cloths were varied between 1, 2, 3, and 4 weeks. Four different buffers were used for extraction of parallel-sampled cloths, and the allergen yields in the extracts were compared. Furthermore, the influence of storage of cloths at room temperature for 2–629 days, or storage of extracts at -80°C for 7–639 days on the outcome of allergen quantification was analyzed. In addition, allergen yields after storage of extracts for 1.5 years at either -80°C or -20°C were compared.

METHODS

Sampling and extraction of settled dust

About 255 EDCs with either four ($n = 92$) or two polyester cloths ($n = 163$) from Zeeman (Alphen aan de Rijn, The Netherlands) with a sampling area on each cloth of 209 cm^2 were placed in households ($n = 125$) or day-care centers ($n = 130$) in a height of about 1.8 m.

The standard procedure for sampling, extraction, and storage was as follows: After 14 days of sampling,

EDCs were closed, put into Ziploc bags and returned to the laboratory. The bags were stored in a freezer at -20°C overnight to eliminate mite proliferation on the cloths and then stored at room temperature until extraction a few days later. The cloths were transferred to 50 ml conical polypropylene tubes and extracted in 15 ml phosphate-buffered saline (PBS, 8.1 mM Na_2HPO_4 , 1.5 mM KH_2PO_4 , 137 mM NaCl, 3 mM KCl, pH 7.4) with 0.05% Tween 20 by rotation for 1 h at room temperature. After removing the cloths, the tubes containing the extraction solution were centrifuged at $3000\times g$ for 15 min. The pellets were discarded, and the supernatants were transferred and stored in aliquots at -80°C . In order to examine how method variations influence allergen yields, several experiments were performed. In the following part, only sample numbers and the deviations from the standard procedure are reported.

Study of reproducibility and duration of sampling

Three cloths per EDC of 36 EDCs were extracted and analyzed in parallel to test reproducibility of sampling. The 36 EDCs had been placed for 14 days in day-care centers ($n = 24$) or households ($n = 12$) without cats or dogs.

In one experiment, the deployment times of the cloths were varied. In 20 households (six with dogs, six with cats) EDCs that contained four cloths were placed. One cloth was transferred to the polypropylene tube after 1 week of sampling, one after 2 weeks of sampling, one after 3 weeks of sampling and one after 4 weeks of sampling. The cloths were stored at -20°C overnight and then at room temperature until extraction at the same time. In addition, all allergen measurements of samples from same EDCs were performed in parallel.

Extraction buffer study

In one experiment, the buffers used for extraction were varied. Twenty-eight EDCs that contained four cloths were placed in different rooms of day-care centres ($n = 26$) and households (one with dog, one with cat) for 14 days. The cloths were extracted with four different buffers: PBS, borate-buffered saline (BBS, 10 mM $\text{Na}_2\text{B}_4\text{O}_7$, 150 mM NaCl, pH 8.0) with or without the addition of 0.05% Tween 20, and PBS without Tween 20. All allergen measurements of samples from same EDCs were performed in parallel.

Storage study

EDC folders were placed in day-care centers or private homes for 14 days in the years 2010–2012. One cloth was analyzed for dog, cat, and domestic mite (DM) allergens immediately after sampling. In 170 EDC folders in which the first cloth was positive for dog allergen, a second parallel sampled cloth was selected and extracted 2–629 storage days later. Then, an aliquot of the first extract was analyzed in parallel to the extract of the second cloth for the six allergens. The extracts of the second cloths were stored for 1.5 years either at -20°C or -80°C and then analyzed again for the six allergens.

Out of the 170 EDCs, 80 were sampled in day-care centers and 91 in households. Among the latter, 70 households owned dogs and 13 households owned cats.

Allergen quantification with fluorescence enzyme immunoassays (FEIA)

The DM immunoassay is based on rabbit antibodies to *Dermatophagoides farinae* allergens and detects antigens from several DM species (Sander *et al.*, 2012). The *Dermatophagoides pteronyssinus* (Dp) and *Tyrophagus putrescentiae* (Tp) immunoassays detect in preference antigens from their name-giving immunogens and are developed as described previously (Zahradnik *et al.*, 2009; 2011a). The cat, dog, and mouse antibodies and standards were purchased from Indoor Biotechnologies Ltd. (Charlottesville, VA, USA) and detect the main allergens Fel d 1, Can f 1, and Mus m 1. Thus, the kits EL-FD1 and EL-MM1 were used for Fel d 1 and Mus m 1, respectively. For detection of Can f 1, the monoclonal antibody 6E9 was biotinylated with a 33 molar excess of Biotin- ϵ -Aminocaprylic-N-Hydroxysuccinimid ester (Roche, Mannheim, Germany), and the monoclonal antibody 10D4 was used as capture antibody. All assays were performed as amplified FEIAs using the streptavidin-peroxidase conjugate Poly-HRP80-SA (Fitzgerald, Concord, MA, USA) and the fluorogenic substrate Quanta blue (ThermoScientific, Rockford, IL, USA). Sample concentrations were calculated by interpolation of relative fluorescence units on a four-parameter fitted standard curve using Softmax Pro 5.4.1 (Molecular Devices, Sunnyvale, CA, USA). The lower levels of detection (LOD) for the amplified FEIAs were 0.05 ng ml^{-1} for DM, 0.1 ng ml^{-1} for Tp and Dp, and 0.01 ng ml^{-1} for Fel d 1, Can f 1, and Mus m 1. For samples in the measuring range of FEIAs the mean interassay coefficients of variation (CV) were about 10%.

Statistical analyses

In the reproducibility study, the mean coefficient of variation of measurements above LOD from cloths in same EDCs was calculated with Microsoft Excel 2010. Pearson correlations between allergen loads on the cloths in same EDC were calculated on log-transformed values with Graph Pad Prism 5.04 (GraphPad Software, Inc., La Jolla, CA, USA).

In the sampling duration study, the sum of allergen loads on the four cloths was calculated for each EDC in which all cloths had allergens above the detection limit. Then, the allergen levels at 0–4 weeks of sampling in relation to 1/5 of this sum (allergen sampled at mean during 2 weeks) were calculated as a kind of normalization. Of these relative allergen levels, means and confidence intervals were calculated for each allergen and time point, and graphs were plotted with Graph Pad Prism.

To describe the variables, medians, interquartile ranges, geometric means, and the corresponding 95% confidence intervals are presented. In case of observations below LOD, the upper bounds of median and quartiles were calculated (Lotz *et al.*, 2013). In these cases, the geometric mean and confidence interval were estimated applying a Tobit regression model (Tobin, 1958) on the log-transformed values with no independent variables besides the intercept, assuming a log-normal distribution of the data. The statistical analysis was performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). To model the effect of buffer and detergent used for extraction on the measured allergen concentration, hierarchical linear models with two levels (1: extracted sample, 2: EDC) were estimated for each allergen. The independent variable was the log-transformed allergen concentration and the dependent variables were buffer (PBS or BBS) and detergent (0.05% Tween or no Tween). Similarly, the effect of EDC-cloth storage duration before extraction was modeled with hierarchical linear models with two levels (1: extracted sample, 2: EDC) for each allergen. The independent variable was again the log-transformed allergen concentration, and the dependent variable was time of cloth storage (per 30 days). In the same manner, the effect of extract storage duration was modeled with two levels (1. aliquot, 2. extracted sample) with time of extract storage duration (per 30 days) as dependent variable. To account for measurements below LOD, the parameters of all

hierarchical models were estimated with a maximum likelihood approach using the expectation maximization algorithm as described by Vaida and Liu (2009). The estimations of the models were done with the software R (R Core Team, 2015).

To compare allergen concentrations after storage at -20°C and -80°C , a paired Prentice–Wilcoxon test was applied (Kalbfleisch and Prentice, 1980) adopting the supplied SAS macro of Lashley *et al.* (2000). For visual inspection, a Bland–Altman plot of the allergen concentrations of DM after storage at -20°C and -80°C is presented. To incorporate the observations below LOD, a multiple imputation approach was applied following the basic idea described by Little and Rubin (2002) and Lubin *et al.* (2004). It was assumed that the paired observations are taken from a bivariate lognormal distribution. Using a bootstrap approach, the parameters of the distribution were estimated with maximum likelihood (Lyles *et al.*, 2001), the censored observations were multiple imputed, and the reference lines (bias line and limits of agreement) were estimated. Observations below LOD were symbolized with five imputed values each. The estimation was done with the software R (R Core Team, 2015). All graphs were calculated by Graph-Pad Prism.

RESULTS

Sampling

Allergen levels were analyzed to determine their consistency between EDC cloths within the same EDC folder. Therefore, three cloths per EDC of 36 EDCs were extracted and analyzed in parallel. The allergen loads were significantly ($P < 0.0001$) correlated and ranged from correlations of 0.906 to 0.986. However, the mean coefficients of variation varied from 21 to 38.8% (Table 1). Also the allergen loads on cloths in the experiment with varied deployment times were in most cases (with the exception of Tp) significantly correlated ($P < 0.05$) and increased with deployment time. More samples exceeded the detection limits, and medians and geometric means were higher after longer sampling periods. To analyze the relative increase over time, only EDC with all allergen values above detection limit were used. In case of DM, 18 out of 20 EDCs fulfilled this criterion, whereas for Fel d 1 and TP only 7 out of 20 EDC contained exclusively cloths with all allergen yields above detection limit.

Table 1. Sampling reproducibility and influence of sampling duration on measured allergen concentrations on EDC cloths

	Can f 1	Feld 1	Mus m 1	Domestic mite	Tyrophagus putrescentiae	Dermato- phagoides pteronyssinus
Sampling reproducibility						
Number of paired samples (<i>n</i>)	3*36	3*36	3*36	3*36	3*36	3*36
Measurements <LOD [<i>n</i> , (%)]	38 (35.2%)	12 (11.1%)	20 (18.5%)	4 (3.7%)	28 (25.9%)	46 (42.6%)
Mean coefficient of variation [%]	24.3%	37.4%	20.8%	37.8%	35.7%	33.2%
Range coefficient of variation [%]	7–66%	8–92%	3–55%	4–90%	2–107%	2–72%
Mean Pearson corr. coefficient	0.906	0.915	0.986	0.970	0.968	0.965
Sampling duration						
Number of paired samples (<i>n</i>)	4*20	4*20	4*20	4*20	4*20	4*20
Mean Pearson corr. coefficient	0.978	0.983	0.838	0.738	0.251	0.836
Measurements <LOD [<i>n</i>, (%)]						
After 1 week of sampling	11 (55%)	12 (60%)	11 (55%)	2 (10%)	12 (60%)	7 (35%)
After 2 weeks of sampling	10 (50%)	12 (60%)	9 (45%)	1 (5%)	7 (35%)	8 (40%)
After 3 weeks of sampling	8 (40%)	9 (45%)	7 (35%)	1 (5%)	3 (15%)	5 (25%)

Table 1. Continued

	Can f 1	Feld 1	Mus m 1	Domestic mite	Tyrophagus putrescentiae	Dermato- phagoides pteronyssinus
After 4 weeks of sampling	6 (30%)	9 (45%)	6 (30%)	0 (0%)	3 (15%)	3 (15%)
Median (IQR) [ng cloth⁻¹]						
After 1 week of sampling	<0.15 (<0.15–14.72)	<0.15 (<0.15–7.27)	<0.15 (<0.15–0.24)	6.16 (2.24–17.95)	<1.50 (<1.50–4.05)	1.94 (<1.5–5.58)
After 2 weeks of sampling	<0.33 (<0.15–20.93)	<0.15 (<0.15–18.22)	0.20 (<0.15–0.32)	20.29 (3.59–32.99)	3.41 (<1.50–6.41)	2.66 (<1.5–7.72)
After 3 weeks of sampling	0.59 (<0.15–24.45)	0.26 (<0.15–19.96)	0.22 (<0.15–0.46)	19.25 (4.10–39.11)	6.50 (1.98–10.30)	4.18 (1.55–8.69)
After 4 weeks of sampling	0.62 (<0.15–25.13)	0.17 (<0.15–38.54)	0.35 (<0.15–0.49)	48.55 (10.49–78.57)	6.26 (2.32–13.78)	4.62 (2.13–11.14)
GM (95% CI) [ng cloth⁻¹]						
After 1 week of sampling	0.13 (0.01–1.54)	0.05 (0.00–1.11)	0.12 (0.06–0.24)	6.11 (3.06–12.21)	1.14 (0.46–2.77)	2.30 (1.14–4.65)
After 2 weeks of sampling	0.24 (0.03–2.29)	0.05 (0.00–1.73)	0.17 (0.09–0.32)	11.44 (5.88–22.25)	2.63 (1.58–4.39)	2.40 (1.05–5.49)
After 3 weeks of sampling	0.55 (0.09–3.52)	0.25 (0.02–2.69)	0.23 (0.12–0.46)	13.13 (6.73–25.63)	4.56 (3.00–6.93)	3.67 (1.83–7.38)
After 4 weeks of sampling	0.94 (0.21–4.20)	0.28 (0.02–3.55)	0.30 (0.16–0.57)	24.91 (13.26–46.80)	5.42 (3.42–8.60)	5.37 (2.74–10.53)

LOD, limit of detection; IQR, interquartile range; CI, confidence interval. Correlation coefficients with $P < 0.05$ are printed in boldface.

Out of 9 EDCs with all cloths positive for Can f 1, six derived from households with dogs. Out of 7 EDCs with all allergens positive for Fel d 1, six were from households with cats. Furthermore, the allergen amount on different EDCs varied up to more than two orders of magnitude, e.g. Fel d 1 in homes with or without cats. Therefore, as a type of normalization, for each EDC relative allergen levels after 1–4 weeks of sampling were calculated and used for graphical analysis (Figure 1). The graphs show more or less linear increases with doubling allergen levels by doubling sampling durations.

Extraction

Parallel sampled cloths were extracted in four different buffers to examine the influence of PBS, BBS, and the addition of Tween 20 on the measured allergen amount (Table 2). Medians and geometric means of allergen concentrations were significantly higher, and fewer samples below detection limits were observed when Tween 20 was used during extraction. Without addition of Tween, only one sample was positive for Fel d 1, and cloths from only 11 places were positive for Can f 1. The effect estimates if extractions were performed without this detergent indicated allergen

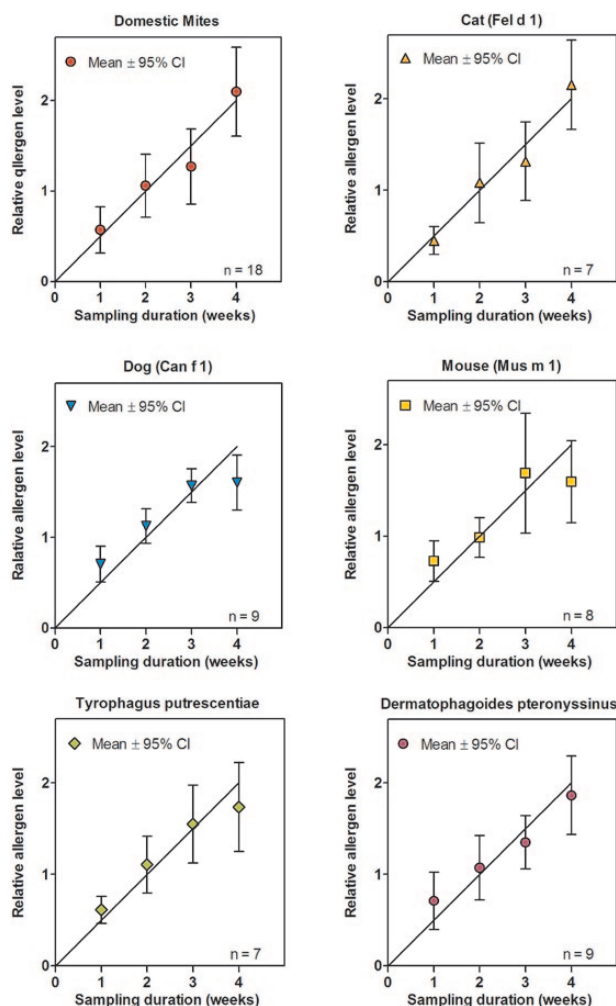


Figure 1 Relative allergen increase with sampling duration. Only sampling series with allergen values above detection limit were used to calculate the relative increase after 1, 2, 3, and 4 weeks of sampling. Shown are the means and confidence intervals at the time points for each allergen and the lines of the theoretically expected increase in case of doubling allergen levels by doubling sampling duration.

Table 2. Effects of buffer and detergent used for extraction on measured allergen concentrations

	Can f 1	Fel d 1	Mus m 1	Domestic Mite	Tyrophagus putrescentiae	Dermatophagoides pteronyssinus
Number of paired samples (<i>n</i>)	4*28	4*28	4*28	4*28	4*28	4*28
Measurements < LOD [<i>n</i> , (%)]	45 (40.2%)	62 (55.4%)	46 (41.1%)	8 (7.1%)	46 (41.1%)	75 (67.0%)
PBS + 0.05% Tween	4 (14.3%)	3 (10.7%)	3 (10.7%)	0	6 (21.4%)	8 (28.6%)
BBS + 0.05% Tween	3 (10.7%)	4 (14.3%)	13 (46.4%)	1 (3.6%)	7 (25.0%)	15 (53.6%)
PBS	21 (75.0%)	28 (100 %)	12 (42.9%)	3 (10.7%)	15 (53.6%)	24 (85.7%)
BBS	17 (60.7%)	27 (96.4%)	18 (64.3%)	4 (14.3%)	18 (64.3%)	28 (100 %)
Median (interquartile range)						
Allergen concentration (ng cloth ⁻¹) after extraction with						
PBS + 0.05% Tween	0.93 (0.41–2.26)	1.78 (0.81–3.69)	0.52 (0.39–0.91)	9.75 (5.40–20.00)	4.65 (2.58–8.21)	2.58 (<1.48–4.48)
BBS + 0.05% Tween	1.08 (0.39–2.60)	1.66 (0.50–3.42)	0.19 (<0.15–0.47)	10.51 (3.68–25.06)	3.16 (<1.53–6.27)	<1.50 (<1.50–3.00)
PBS	<0.15 (<0.15–<0.19)	<0.15 (<0.15–<0.15)	0.18 (<0.15–0.43)	3.72 (1.89–11.84)	<1.50 (<1.50–3.17)	<1.50 (<1.50–<1.50)
BBS	<0.15 (<0.15–0.52)	<0.15 (<0.15–<0.15)	<0.15 (<0.15–0.33)	4.76 (2.04–12.08)	<1.50 (<1.50–3.65)	<1.50 (<1.50–<1.50)
Geometric mean (95% CI)						
Allergen concentration (ng cloth ⁻¹) after extraction with						
PBS + 0.05% Tween	0.83 (0.53–1.31)	1.57 (0.94–2.61)	0.54 (0.41–0.72)	9.97 (6.68–14.87)	4.60 (3.20–6.63)	2.48 (1.77–3.47)

Table 2. Continued

	Can f 1	Fel d 1	Mus m 1	Domestic Mite	Tyrophagus putrescentiae	Dermatophagoides pteronyssinus
BBS + 0.05% Tween	0.86 (0.53–1.31)	1.25 (0.71–2.21)	0.18 (0.11–0.29)	8.87 (5.50–14.29)	3.04 (2.06–4.49)	1.27 (0.68–2.38)
PBS	0.03 (0.00–0.20)	*	0.19 (0.11–0.30)	4.26 (2.53–7.17)	1.30 (0.67–2.52)	0.80 (0.40–1.60)
BBS	0.10 (0.04–0.27)	0.01 (0.00–1.81)	0.10 (0.05–0.21)	4.61 (2.66–7.98)	0.93 (0.39–2.23)	*
0.05% Tween (Ref.) versus no Tween						
Effect estimate exp(β)	0.14	0.01	0.48	0.49	0.40	0.15
Confidence interval	(0.10–0.19)	(0.006–0.02)	(0.40–0.59)	(0.40–0.59)	(0.31–0.50)	(0.09–0.26)
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PBS (Ref.) versus BBS						
Effect estimate exp(β)	1.26	0.90	0.47	0.99	0.73	0.55
Confidence interval	(0.89–1.63)	(0.63–1.29)	(0.38–0.57)	(0.81–1.20)	(0.58–0.92)	(0.37–0.83)
P value	0.22	0.58	<0.0001	0.91	0.0077	0.0045

LOD, limit of detection; Ref., Reference.

*Could not be calculated all observation <LOD, P values <0.01 are printed in boldface.

losses for Fel d 1 of about –99%, for Can f 1 –86%, for Dp –85%, Tp –60%, Mus m 1 –52%, and for DM –51%. Comparing the influence of borate versus phosphate buffers on the allergen amounts, only for the Mus m 1, Dp, and Tp significant effects were observed. Using borate instead of phosphate buffer, for Mus m 1 a loss of about –53%, for Dp –45%, and for Tp –27% was indicated by calculation of the effect estimates.

Storage

In a previous experiment (data not shown), it was observed that Can f 1 amounts decreased by more than 50% after approximately 18 month storage of EDC cloths before extraction. For analysis of the influence of cloth storage duration on the allergen yields, from 170 EDC folders, a second parallel sampled cloth was extracted 2–629 days later. Both extracts were analyzed in parallel with the six FEIAs. Figure 2 documents a cumulative loss of dog allergen values on EDC cloths with increasing storage time at room temperature. Using a linear mixed model, a significant loss of 4.81% Can f 1 for every 30 days of storage was calculated (Table 3). A loss of allergen during cloth storage was exclusively observed for the dog allergen, for Tp even minimal higher allergen values after storage were observed. Storage of extracts at –80°C significantly decreased DM results (1.16% for every 30 days) while no significant changes for Can f 1 or Fel d 1 yields were found. Extracts stored for 1.5 years either at –20°C or at –80°C were measured in parallel in same assays. Significantly higher values were obtained for DM and Fel d 1 if the aliquots were stored at –20°C compared

to storage at –80°C (Table 3). This difference was not dependent on DM concentration (Figure 3).

DISCUSSION

The use of amplified FEIAs, either based on antibodies from Indoor Biotechnologies against single mammalian allergen molecules or on polyclonal antibodies against antigens from DM species, enabled quantification of allergens in the majority of samples from homes and day-care centers. In contrast, single house dust mite allergens were below the limit of detection on EDCs in previous experiments (Krop *et al.*, 2014; Kilburg-Basnyat *et al.*, 2015).

Allergen levels on parallel sampled cloths in EDCs were closely correlated. However, mean CV of allergen levels on parallel sampled cloths were with 21–38% much higher than the mean inter-assay CV of FEIAs (ca. 10%). Due to the fact that allergens are transported on particles that follow random Poisson distribution, at low allergen concentrations high CV values in airborne samples are the consequence (Doekes *et al.*, 2014).

Longer deployment increased geometric means for all allergens, although for individual EDCs the allergen amount on longer exposed cloths did not follow the expected increase in all cases, most probably due to stochastic distribution of allergen particles. Overall, a more or less proportional increase with doubled allergen level for doubled sampling time on the electrostatic cloths was observed. Thus, over a four weeks range allergen levels might be interpolated which enables an adjustment in studies when participants

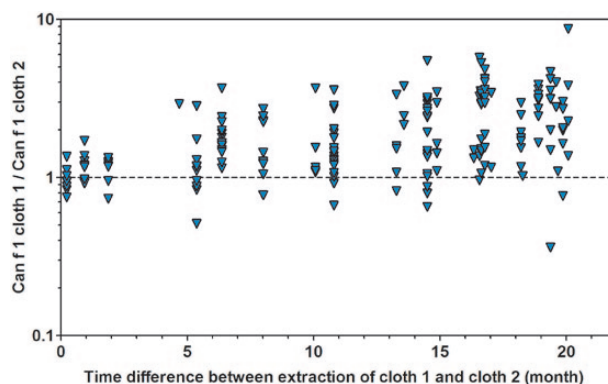


Figure 2 Influence of cloth storage duration on Can f 1 yields. Parallel sampled cloths were either extracted shortly after sampling (cloth 1) or up to 629 days later after storage at room temperature (cloth 2). The relations between Can f 1 concentrations in both extracts were calculated only for values above the detection limit.

Table 3. Effects of EDC-cloth storage duration before extraction and extract storage duration and temperature on measured allergen concentrations

	Can f 1	Feld 1	Mus m 1	Domestic Mite	Tyrophagus putrescentiae	Dermatophagoides pteronyssinus
Cloth storage duration before extraction						
Number of paired samples (<i>n</i>)	2170 = 340	2166 = 332	2158 = 316	2170 = 340	2159 = 318	2158 = 316
Measurements below LOD [<i>n</i> , (%)]	4 (1.18%)	99 (29.82%)	71 (22.47%)	11 (3.24%)	75 (23.58%)	103 (32.59%)
Days of storage: Median (range)	60.5 (2–629)	60 (2–629)	60 (2–629)	60.5 (2–629)	60 (2–629)	60 (2–629)
Estimated change of allergen concentration per 30 days of cloth storage (%) ^a	–4.81%	+1.04%	+0.93%	+0.44%	+1.67%	–0.09%
95% Confidence interval of change ^a	–5.37% to –4.25%	+0.04% to +2.04%	+0.02% to +1.67%	–0.51% to +1.39%	+0.64% to +2.71%	–1.12% to +0.95%
<i>P</i> value ^a	<0.0001	0.0406	0.0130	0.366	0.0014	0.862
Extract storage duration at –80°C						
Number of paired samples (<i>n</i>)	2149 = 298	2191 = 182	—	2150 = 300	—	—
Measurements below LOD [<i>n</i> , (%)]	8 (2.68%)	18 (9.00%)	—	15 (5.00%)	—	—
Days of storage: Median (range)	287 (7–637)	289 (7–637)	—	303.5 (7–637)	—	—
Estimated change of allergen concentration per 30 days of extract storage (%) ^a	+0.75%	–0.28%	—	–1.16%	—	—

Table 3. Continued

	Can f 1	Feld 1	Mus m 1	Domestic Mite	Tyrophagus putrescentiae	Dermato- phagoides pteronyssinus
95% Confidence interval of change ^a	−0.06% to +1.56%	−0.69% to +0.14%	—	−1.47% to −0.86%	—	—
P value ^a	0.068	0.192	—	<0.0001	—	—
Extract storage temperature						
Number of paired samples (<i>n</i>)	2150 = 300	2151 = 302	2151 = 302	2152 = 304	2152 = 304	2150 = 300
Measurements below LOD [<i>n</i> , (%)]	10 (3.33%)	86 (28.48%)	71 (23.51%)	16 (5.26%)	69 (22.70%)	103 (34.33%)
Allergen concentrations after storage at −20°C: Median [ng cloth ^{−1}] Interquartile range [ng cloth ^{−1}]	2.55 (0.74–7.61)	0.87 (<0.16–3.50)	0.39 (<0.16–0.68)	11.29 (3.94–26.04)	4.11 (1.59–8.31)	2.81 (<1.51–5.69)
Allergen concentrations after storage at −80°C: Median [ng cloth ^{−1}] Interquartile range [ng cloth ^{−1}]	2.20 (0.72–7.43)	0.81 (<0.16–3.24)	0.41 (<0.17–0.71)	9.47 (3.86–24.95)	4.37 (1.70–8.63)	2.94 (<1.51–5.90)
P value ^b	0.061	0.0155	0.052	<0.0001	0.6347	0.108

LOD, limit of detection.

^aLinear mixed model with censored response. ^bPrentice–Wilcoxon test for censored paired data. *P* < 0.01 are printed in boldface.

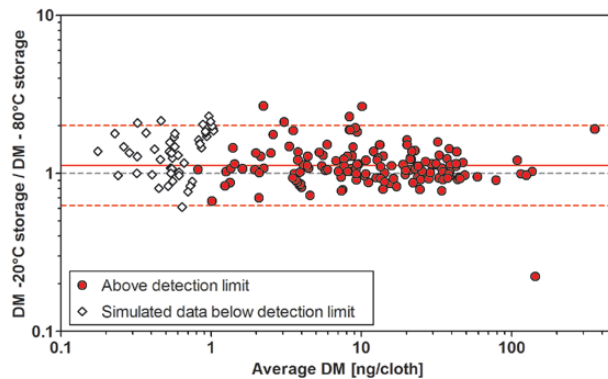


Figure 3 Quotient of the DM value of the extract stored at -20°C and the DM value of the extract stored at -80°C in relation to the average of both values (Bland-Altman Plot). Observations below the detection limit (six after both storage conditions, three at -80°C and one at -20°C) were symbolized with five imputed values. The bias line (solid line at 1.12) and the limits of agreement (dashed lines at 0.63 and 2.01) are indicated.

deviate from the deployment time of the study protocol. In a similar experiment, Kilburg-Basnyat *et al.* (2015) also found an increase of Can f 1 and Mus m 1 over longer sampling periods, but had observed that Fel d 1 load on EDC cloths did not increase significantly. However, they used an extraction procedure for allergens without addition of a detergent. Especially for extracting Fel d 1 from the cloths, the addition of Tween 20 seems to be crucial.

In our experiment with four different extraction buffers, all allergen yields were significantly higher when the buffer contained 0.05% Tween 20 ($P < 0.0001$). The effect estimates ranged from 99% less Fel d 1 and 86% less Can f 1 to 51% less DM for the buffers without Tween 20. A previous comparison had shown that Fel d 1 and Can f 1 values in the amplified FEIA were similar to the ones obtained in MARIA (Sander *et al.*, 2013). Thus, it is likely that differences in extraction procedures are responsible for different outcomes and not differences between FEIA and MARIA, the Indoor multiplex array used by Kilburg-Basnyat *et al.* (2015) for allergen quantification. Therefore, analog to the findings of Noss and Doeke *et al.*, 2010, for endotoxin, addition of Tween 20 is advantageous for extraction of allergens from EDC cloths. In addition, the influence of buffer ions on extraction yields was investigated. Previous extraction experiments of Der p 1 (main allergen from *Dermatophagoides pteronyssinus*) from reservoir dust have shown contradicting results. Siebers (2012) reported higher Der p 1 yields with a borate buffer compared to phosphate or

ammonium bicarbonate buffer. However, Prester *et al.* (2012) found no differences when they compared these buffers for extraction efficiency of Der p 1. In our study, EDC cloths extracted in BBS gave lower yields of Mus m 1 (−53%), Tp (−27%), and Dp (−45%) than EDC cloths extracted in PBS whereas no significant differences were observed for Can f 1, Fel d 1 or DM. Thus, for EDC extraction of allergens PBS seems to be the preferred buffer compared to BBS.

Furthermore, we examined the influence of storage duration on allergen yields of cloths or cloth extracts. Lag times due to limited capacity of the laboratory might be unavoidable especially in huge studies. Knowledge on allergen stability during storage is helpful to optimize study logistics, e.g. to determine whether to prefer cloth or extract storage. Storage of cloths at room temperature decreased the Can f 1 amount by estimated 4.81% loss for every 30 days ($P < 0.0001$). The calculated loss of Can f 1 is approximately 60% for storage for 1.5 years. It has to be kept in mind that allergen quantification with monoclonal antibodies in FEIA (and also MARIA) is based on two epitopes only. If these epitopes are separated by allergen degradation, the allergen will not be detected in monoclonal antibody based sandwich immunoassays. In most previous studies on Can f 1, immunoassays with a combination of monoclonal and polyclonal antibodies had been used (Zahradnik and Raulf, 2014). Therefore, stability in our experiments and also in case of quantifying Can f 1 by MARIA might

differ from previous studies on resistance of Can f 1 to degradation (Cain *et al.*, 1998). Finally EDC extracts were stored in aliquots either at -80°C or at -20°C for 1.5 years. Significant differences were observed for DM ($P < 0.0001$). The Bland Altman analysis showed at mean 12% higher DM values after storage at -20°C compared to DM values after storage at -80°C for 1.5 years. It has been reported previously that fast freezing with slow thawing resulted in more severe damage to proteins due to formation of ice crystals and protein denaturation (Cao *et al.*, 2003). Storage of extracts at -80°C resulted in a loss of 1.16% of DM for every 30 days of storage ($P < 0.0001$). That is a loss of about 19% for 1.5 years of storage at -80°C . DM loss might be partially avoidable if extracts are stored at -20°C . However, storage of frozen aliquots of extracted samples seems to be preferable compared to storage of cloths at room temperature when Can f 1 quantification is planned. Storage of cloths in the freezer had not been tested, but might be also an option in case of sufficient freezer capacity. However, an immediate analysis of samples should be preferred.

CONCLUSION

EDCs can be used as reproducible tools for quantification of the main allergens from cat, dog and mouse, and different antigens from DM species in indoor environments. As allergen levels increase proportionally over a four weeks period of sampling, EDC results could be interpolated to adjust for deviations from the study protocol or to compare studies with different sampling duration. For allergen extraction we recommend PBS with 0.05% Tween 20. Storage duration of cloths and frozen extracts had little influence on allergen levels with the exception of Can f 1 which decreased by 4.81% for every 30 days of cloth storage at room temperature. Further studies are necessary to find out whether instability of Can f 1 epitopes for two-sited monoclonal antibody detection is a general problem for quantification of Can f 1 in dust.

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