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qPCR standard operating procedure for measuring microorganisms in dust from dwellings in large cohort studies

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HIGHLIGHTS

- Pilot study of the qPCR analysis of dust collected by an Electrostatic Collector
- Standard Operational Procedure fulfills feasibility, cost and efficacy criteria.
- Chosen targets were 6 fungi, *Enterobacteria*, *Mycobacteria*, *Streptomyces* and mites.
- With this SOP, dwellings can be classified according to microbial DNA quantity.

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ABSTRACT

The aim of the present study was to assess performance, feasibility and relevance of a Standard Operational Procedure (SOP) for large-scale use in the microbial analysis of children's indoor environments.

We analyzed dust settled on Electrostatic Dust Fall Collectors (EDCs) by using qPCR which targeted 6 molds, 3 bacteria and 1 mite, chosen for their involvement in allergic or inflammatory processes. Six types of commercialized electrostatic wipes were tested for their releasing capacity of fungal DNA from fungal spores captured by the wipes. Specificity, repeatability and detection limits of the qPCR procedure were tested using calibrated microbial suspensions. The feasibility and relevance of this sampling and analysis method were assessed in a 75-home pilot study.

Our result showed that one specific make of wipe was more effective than the others in releasing fungal DNA. qPCR procedure showed good repeatability. The quantification limit was about 5 fg DNA/μL for all species except *Penicillium chrysogenum* (0.5 fg DNA/μL) and *Dermatophagoïdes pteronyssinus* (10 fg DNA/μL). No cross-reactivity was observed.

DNA concentrations in the 53/75 homes participating in the pilot study were between 0 and 24 625, 0 and 69 738 equivalent cells per cm^2 for the fungi and bacteria, and between 0 and 1 equivalent mites per cm^2 for *D. pteronyssinus*. Using the SOP described, we were able to classify the 53 dwellings from the least to the most contaminated according to the quantity of DNA measured for each species.

Our SOP measured fungi, bacteria and mites using a cost-efficient, discreet and well-accepted sampling method with just one qPCR tool. The whole procedure can be used for microbial analysis in large cohort studies such as the ELFE study ("Etude Longitudinale Française depuis l'Enfance") and could help improve our understanding of the interactions between the environment, allergic diseases and child development.

1. Introduction

Asthma and related atopic diseases are now among the most common childhood diseases in industrialized countries (Asher et al., 2006; Eder et al., 2006). The combined effect of environmental exposure to indoor chemical pollutants and microbial contaminants, and genetic

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predisposition is unknown (Braun-Fährlander et al., 1999; von Mutius, 2007b). A significant difference in mold exposure between non-asthmatic and severely asthmatic populations has also been demonstrated (Reboux et al., 2009; Reponen et al., 2011; Vesper et al., 2008). Some studies have shown that exposure to higher levels of endotoxin protects against allergy (Ownby et al., 2010). Despite extensive research, doubt remains that exposure to indoor allergens causes asthma and allergy, because various studies have presented conflicting evidence, as reported by Arshad after a review of the literature. He concludes that the greatest effect of exposure to indoor allergens (molds, mites, pets, etc.) probably occurs during the postnatal period (Arshad, 2010).

The purpose of the ELFE "Etude Longitudinale Française depuis l'Enfance" project is to establish a nationally representative cohort of 20 000 children to be followed from birth to adulthood using a multidisciplinary approach to characterize the effects of environmental exposure, disease and the socio-economic context on health (Vandentorren et al., 2009). Many factors are known to influence child development and children's health and different working groups have been convened, including a specific group focusing on asthma and allergic respiratory diseases.

To study environmental exposure we suggested using Electrostatic Dust Fall Collector (EDC) (Normand et al., 2009; Noss et al., 2008; Frankel et al., 2012) and analyzing settled dust from the EDCs using qPCR, targeting 6 molds (*Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus versicolor*, *Cladosporium sphaerospermum*, *Penicillium chrysogenum*, *Stachybotrys chartarum*), 3 bacteria (*Enterobacteriaceae*, *Mycobacteria*, *Streptomyces*) and 1 mite (*Dermatophagoïdes pteronissynus*). Literature concerning microbial species involved in allergic diseases is abundant and controversial. The 10 target microorganisms were chosen because they belong to classes or genera that are phylogenetically very distinct, and because they have been implicated in human diseases due to their allergic, toxic or infectious effects.

EDC is a newly developed assessment method that provides an alternative to surface or air sampling in epidemiological studies (Frankel et al., 2012; Tischer and Heinrich, 2013). Previous studies have shown that qPCR quantification of targeted species is an easy and reliable tool for characterizing the homes of allergic patients, and have demonstrated its advantages for studies in homes (Kaarakainen et al., 2009; Reponen et al., 2011; Rintala et al., 2004; Vesper, 2011; Vesper et al., 2007).

In the present study, we assessed the optimization, feasibility and relevance of this sampling and analysis method in a 75-home pilot study with the aim of determining a Standard Operational Procedure (SOP) that can be used in large-scale microbial and allergenic analyses of children's indoor environments; we had the ELFE study specifically in mind.

2. Materials and methods

2.1. Dust collection

2.1.1. Sampling device (EDC)

The EDC consists of commercially available electrostatic wipes described by Noss et al. (2008) set in a plastic case, which the participant opens. The collection involves capturing settled airborne dust via the electrostatic properties of the wipe. The EDC may be considered as a surrogate for the assessment of indoor airborne microbial exposure. The EDC is also easy to use, as it can be sent by mail (Frankel et al., 2012). Wipes were first sterilized in an autoclave (124 °C for 30 min) and stuck on a disinfected (Surfanios™, Anios®, Lille-Hellemmes, France) polypropylene case (resembling a DVD case) with white glue (UHU®, Bühl, Germany).

2.1.2. Wipe material selection

Six different types of commercialized electrostatic wipe cloths, each with different textures, were tested for their capacity to release fungal DNA from the fungal spores captured on them (Table 1). Three wipes of each type were impregnated with two 1 mL suspensions, one of *P. chrysogenum* and one of *S. chartarum* in known concentrations (7×10^5 and 4×10^5 spores/mL respectively) and dried overnight. Each wipe was put in a plastic bag with a washing solution of 20 mL of 0.1% Tween 80 solution (Merck®, Darmstadt, Germany) and shaken for ten minutes in a Stomacher™ (AES®, Combourg, France). Ten (± 0.5) mL of washing solution was then collected.

The qPCR protocol as described below was then applied to the DNA extract from the 2 initial fungal spore suspensions and from the washing solution from the wipe. The capacity of the wipe to release microbial DNA was deduced by comparing the two qPCR results taking into consideration the dilution by the washing liquid volume (20 mL). Tests were also performed using volumes of washing liquid of 40 mL.

2.2. qPCR protocol

2.2.1. DNA extraction

Rapid DNA extraction, chosen for its simplicity, cost and efficiency (Haugland et al., 2002), was performed as previously described by Keswani et al. with the following changes (Keswani et al., 2005).

Extraction was carried out using an initial sample volume of 200 μL of fungal spore suspension or 200 μL of washing solution from the wipe. The samples were placed in 2 mL conical-bottom screw-cap tubes (MagNA Lyser Green Beads, Roche Applied Science®, Mannheim, Germany) containing 1.4 mm diameter ceramic beads and 200 μL of brain–heart infusion (BBL™, Becton Dickinson®, Sparks, NJ, USA). The tubes were shaken in a MagNA Lyser Instrument (Roche Applied Science®, Mannheim, Germany) three times for ten seconds at the maximum speed with one minute in the cooling block (4 °C). The tubes were then heated in a boiling water bath for ten minutes. The samples were placed on ice for ten minutes, followed by centrifugation for two minutes at 8000 rpm at room temperature. The supernatant above the beads was removed and stored at 4 °C for subsequent qPCR analysis.

A sterile distilled water sample was used as a negative control in each extraction series.

Table 1
Different makes of wipes and their capacity to release microbial DNA. Wipes were impregnated with suspension of *P. chrysogenum* and *S. chartarum*, then washed as described in "Materials and methods". The qPCR protocol was applied to the DNA extract from the initial suspension and from the washing solution. Each experiment was done in triplicate. Quantitative results were expressed by determining the detection threshold, or quantification cycle (Cq), that marked the cycle at which fluorescence of the sample became significantly different from the baseline signal. Thus, the higher the Cq, the smaller the amount of DNA in the sample.

	Make of wipes	*Penicillium chrysogenum* (*Pc*)	*Stachybotrys chartarum* (*Sc*)	Recovery efficiency (%)	
Fungal suspension (sp/mL)		7×10^5	4×10^5	(*Pc*)	(*Sc*)
Cq (cycles)		32.3	23.8		
Cq from the EDC washing liquid (mean Cq/SD)	Wipes 1 (Apta top budget)	36.8/1.1 4.1×10^4 sp/mL	28.5/0.8 2×10^4 sp/mL	5.9%	5%
	Wipes 2 (Dia)	36.9/0.9 3.9×10^4 sp/mL	29.1/0.6 1×10^4 sp/mL	5.6%	2.5%
	Wipes 3 (Casino)	36.9/1.7 3.9×10^4 sp/mL	30.5/1.4 5×10^3 sp/mL	5.6%	1.3%
	Wipes 4 (Casino-First)	37.1/1.1 3.4×10^4 sp/mL	30.8/1.4 4×10^3 sp/mL	4.9%	1%
	Wipes 5 (Wiffer)	37.8/1.2 2.2×10^4 sp/mL	29.1/0.9 1×10^4 sp/mL	3.1%	2.5%
	Wipes 6 (Super U)	38.6/0.7 1.3×10^4 sp/mL	30.1/0.9 7×10^3 sp/mL	1.9%	1.8%

sp/mL: spores per milliliters; Cq: quantification cycle; SD: standard deviation.