Protocols for sampling and analyses of biological indoor air pollutants: allergens and endotoxin

Technical Report – July 2016

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Summary

1.Introduction

In recent decades the prevalence of allergic diseases has increased around the world [1] and the genetic changes can not explained this phenomenon [2]. Exposure to environmental pollutants or microorganisms especially in air (both indoors and outdoors) has in fact been identified as the main cause of many common ailments along with allergies (and chemical sensitivities) [3]. The main indoor biological air pollutants are: dust mite allergens (Dermatophagoides pteronyssinus and farina), cat and dog allergens and endotoxin. Dust mites aeroallergens feed off dead skin cells from humans and often become a major component of indoor dust. Dust mites, like other biological contaminants, contribute to poor indoor air quality (IAQ). *Dermatophagoides* allergens play an important role in inducing IgE-mediated sensitization and the development of bronchial hyperresponsiveness (BHR) and asthma [4]. Also allergies to pets are common, especially among people who have other allergies or asthma. Cat allergies are about twice more common than dog allergies [5]. For about 20 to 30 percent of people with asthma, cat contact can trigger a severe asthma attack [6]. The Lipopolysaccharide (LPS) is a toxic glycolipid presents in the outer membrane of Gram-negative bacteria. It is a signal molecule that actives the innate immune response.

Recent studies demonstrated that exposure to high endotoxin levels in indoor environments may cause inflammatory reactions, systemic effects and irreversible chronic bronchitis [7].Conversely exposure to a lower level of endotoxin in domestic environments during childhood may decrease the risk of allergic sensitization and disorders in preschool and school-age children [8] (Braun-Fahrlander et al., 2002). These observations correspond to the hygiene hypothesis, which suggests that early life exposure to infections and microbes reduces the risk of allergic diseases [9].

This protocol describes the methods used to perform the sampling of indoor dust for analyses of biological pollutants as well as endotoxin and allergens for air quality evaluation. These sampling campaigns are quick and easy to perform; indoor floor dust and dust from other surfaces will be collected using vacuum cleaners with a Indoor Dustream™ Collector and filters (Indoor Biotechnologies). All indoor biologiacal pollutants were purified and analyzed by Chromogenic LAL test for endotoxin concentration evaluation and by monoclonal ELISA assays to identify the main allergens as well as Dermatophagoides pteronyssinus major allergen (Der p1) and cat allergen (Fel d1) in indoor environments.

2. Dust sampling protocol definitions

"Dust Sampling"	Collection of settled dust from floor and above floor level using the Indoor Biotechnologies Dustream collector and a vacuum cleaner
"Dustream Collector"	Consist of a plastic adaptor that can be connected to most of the standard vacuum cleaners and a upper part used for vacuuming the dust; between the adaptor and the upper part is located the Dustream filter (Fig.1).
"Dustream filter"	A nylon cylindrical filter that will be inserted into the Dustream collector
"Ziploc bag"	Consist of a small plastic bag with a re-sealable zipper used for the storage of a single filter containing the dust sample
Fieldwork Booklet	Documentation sheet on which the relevant information concerning the study schools and the sampling must be noted.
Sample ID code sticker	Sticker with a pre-printed ID code for samples identification

2.1 Materials and equipment needed

- Vacuum cleaner used in all study centers will be provided of electricity plug-in that fit to local electricity sources
- A Dustream filter per room
- Re-sealable Ziploc bags for storing filter containing the dust samples (a single filter per ziploc bag)
- Predefined ID code stickers
- Blank stickers
- Timer
- Calculator
- Measuring tape
- Fieldwork Booklet
- Clean paper wipes
- Sterile distilled water for cleaning of Dustream collector
- 70% ethanol for cleaning of Dustream collector
- Pens
- Laboratory gloves
- Laboratory coats

2.2 Dust sampling procedure

The Indoor-Biotechnologies Dustream ™ collector is one of the system used for dust sampling in indoor environments. It is an easy and quick method to collect dust from floors and furniture.

The following text describes the procedure:

1. Connect the vacuum cleaner to electric plug
2. Insert the plastic filter into the Dustream™ collector and attach the collector to the vacuum cleaner (Fig.1-2)

Fig.1 The Indoor-biotechnologies Dustream™ Collector.

Consist of a plastic adaptor piece that can be connected to most of the standard vacuum cleaners and a upper part, the collector, used for vacuuming the dust; between the adaptor and the upper part is located the Dustream filter.

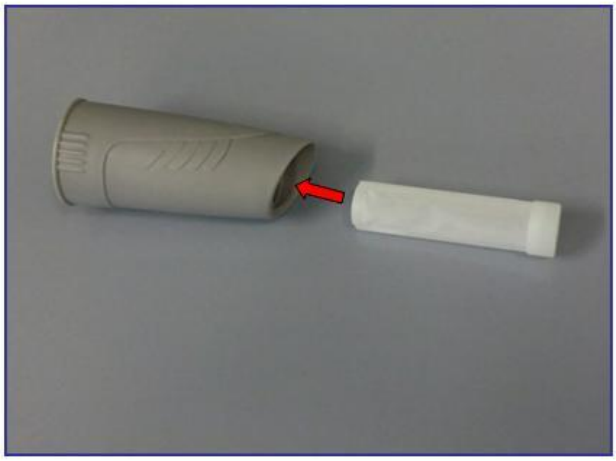
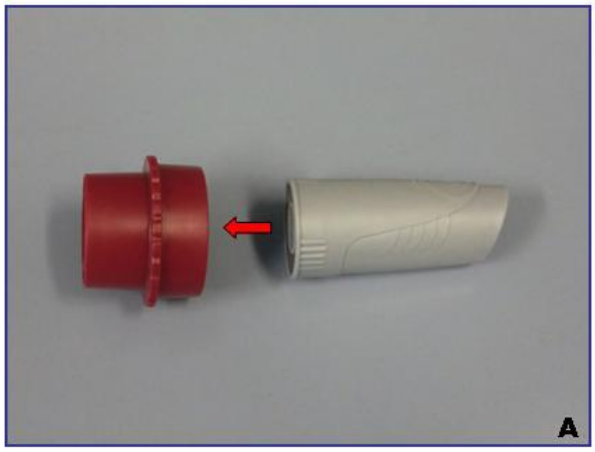


Fig.2 The Dustream™ Collector+ filter

Insert the plastic filter into the Dustream™ Collector (the closed side of the filter facing the inside of collector). Attach the collector to the vacuum cleaner tube

3. If the collector does not fit the vacuum cleaner tube, attach the adaptor piece (Fig.3) to the collector (use the side of the adaptor which fits your vacuum cleaner).



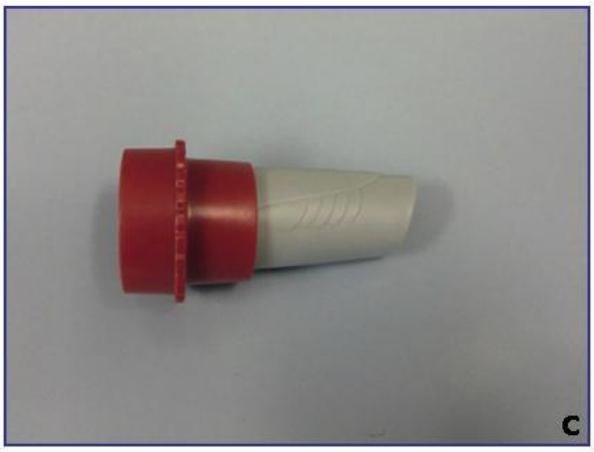


Fig.3 The Adaptor piece.

A. If the collector does not fit the vacuum cleaner tube, attach the adaptor piece to the collector. **B-C.**The adaptor pice can be used from both directions

4. Turn on the vacuum cleaner and vacuum six separate areas (1 square meter per area) on the floor (corners and central part of the room) for 20 second each (total sampling time = 2 minute).
5. Vacuum six separate areas (1 square meter per area) on parts above the floor (desks, doors, chairs, windows fill and so on) for 20 seconds each (total sampling time= 2 minutes).

6. Remove the filter containing the dust sample (Fig.4) and place it in a small Ziploc bag (Fig.5) and identify the sample with the appropriate Sticker with the pre-printed ID code.
7. Rinse the dustream collector with water, dry with a clean paper wipes and place a clean filter into the collector for a new sampling.

Fig.4 Immediately after the sampling remove the dustream filter from dustream collector and to transfert its into the Ziploc bag. Be careful not to touch the filter without gloves

Fig.5 The dust sample must be marked with the the pre-defined ID code stricker on the Ziploc bag

In order not to damage allergens stability and not to allow the bacteria proliferation, samples must be immediately stored on ice, away from heating source and direct sunlight. For long term storage the samples should be stored at -20°C.

To minimize contaminations from others sources some precautions are requested:

- Make sure the cleaning of the room happens not just before the sampling,

- to keep the dustream collector and the dustream filter in the respective closed plastic bag until the sampling;
- do not touch the filter without gloves;
- to close the dustream filter into the dustream collector immediately after opening of the plastic bag;
- to remove the dustream filter from dustream collector immediately after the sampling and to transfert its into the Ziploc bag;
- to mark with the pre-defined ID code by placing an ID code stricker on the Ziploc bag;
- to wash the Dustream collector with sterile distilled water, with 70% ethanol and to dry the collector;
- to change the dustream filter before a new sampling;
- to set the switch on vacuum cleaner with tape at maximum power and make sure the air-outlet is closed to guarantee ideal suction
- to remove the vacuum cleaner internal filters before the sampling, to reduce its overheating.

3. Biological pollutants extraction procedure

In order to extract biological pollutants 100 µg of dust are resuspende in 2 ml of PBS-T (phosphate buffered saline, pH 7.4, 0.05% Tween 20) in pyrogen-free water. For samples between 20mg and 100mg is added the proportional amount needed. Samples <20mg are not processed. Before the samples are mixed using a vortex and after using a laboratory rocker at room temperature (20-25C°) for 2 hours. Samples were centrifuged at 2,500 rpm for 20 minutes at 4°C.

The supernatant (approximately 1.5ml) were removed with a Pasteur pipette, and transferred in a clean 2 ml tube. The dust pellet was discarded.

The sample tubes were centrifuged at 2,500 rpm for 10 minutes at 4°C again. The supernatants were removed and divided in aliquots (~200 µl each).

The extracts were stored at -20°C in a freezer vial with sample number or relevant code clearly labeled for analysis of allergen and endotoxin content.

4. Endotoxin evaluation: QCL-1000^{TM} Limulus Amebocyte Lysate (LAL)-microplate method

The QCL-1000TM Limulus Amebocyte Lysate (LAL) test is an international validate quantitative test for Gram-negative bacterial endotoxin or Lypopolysaccarides (LPS). Gram-negative LPS catalyzes the activation of a proenzyme in *Limulus* extract. The endotoxin concentration catalyze the rate of the reaction. In fact the activated enzyme catalyzes the release of p-nitroaniline (pNA) producing a yellow color from a colorless substrate. The pNA concentration is measured photometrically at 405–410 nm. The absorbance of the sample is in direct proportion to the amount of endotoxin present and the LPS concentration can be calculated from a standard curve in a range from 0.1 to 1.0 EU /ml.
Protocol is describes below:

1. Pre- equilibrate the microplate at 37°C±1°C in the heating block adapter.

2. While leaving the microplate at 37°C±1°C, carefully dispense 50 µl of samples or standard into the appropriate microplate well. Each series of determination must include a blank plus the four endotoxin standards run in duplicate (0.1 EU/ml, 0.25 EU/ml, 0,5 EU/ml, 1 EU/ml). The blank wells contain 50 µl of LAL Reagent Water instead of samples. All reagent addictions and incubation times are identical.

3. At time=0, add 50 µl of LAL to the first microplate well, or first column of microplate wells if using a multi-channel pipettor and reagent reservoir. Begin timing as the LAL is added. Once the LAL has been dispensed into the microplate wells containing samples or standards, briefly remove the microplate from the heating block adapter and repeatedly tap the side of the plate to facilitate mixing. Return the plate to the heating block adapter and replace cover.

4. At time=10 minutes, add 100 µl of substrate solution (pre-warmed to 37°C±1°C). Pipette the substrate solution in the same manner as in Step 3. Maintain a consistent pipetting rate. Once the substrate solution has been dispensed into all microplate wells, briefly remove the microplate from heating block adapter and repeatedly tap the side of the plate to facilitate mixing. Return the plate to the heating block adapter and replace cover.

5. At time=16 minutes, add 100 µl of stop Reagent (25% glacial Acetic acid). Maintain the same pipetting order as in Step 3 and 4. Once the stop reagent has been dispensed into all microplate wells, remove the plate and repeatedly tap the side of the plate.

6. Read the absorbance of each microplate well at 405-410 nm (Fig.1)

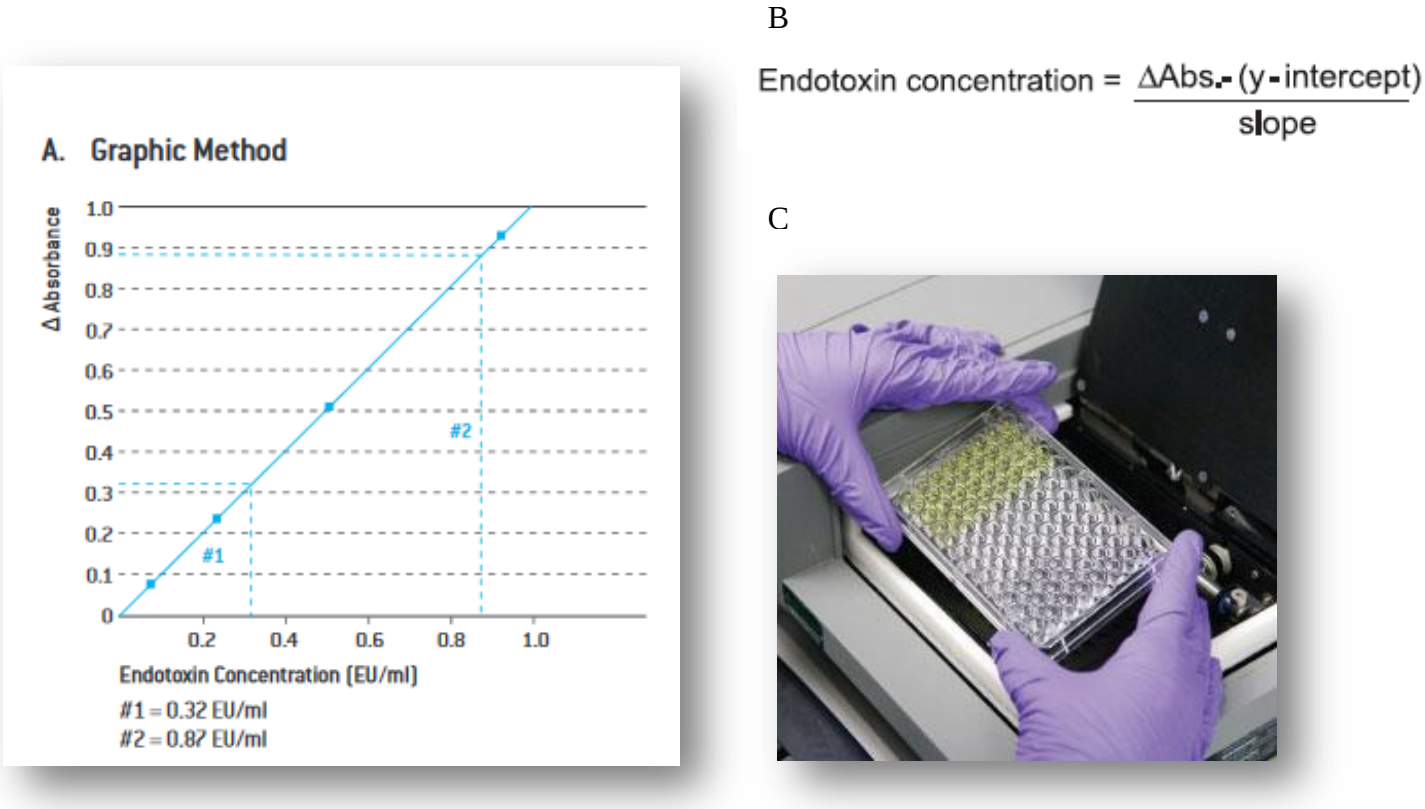


Fig. 1. **A**. The graphic methods for endotoxin determination; **B**. the linear correlation between Absorbance and endotoxin concentration; **C.** a photo of the chromogenic LAL test (Photo courtesy of Lonza-http://www.horseshoecrab.org/med/testing-methods.html)

5. Allergens quantization: ELISA Protocol for Der p 1 and Fel d1

The Der p1 and Fel d1 allergens indoor evaluations were performed using an international validate monoclonal based ELISA assay produced by Indoor Biotechnologies. Protocol is describes below:

1. Dilute the mAb (mAb 5H8 Der p1, mAB 6F9 Fel d1) 1/1000 (i.e.10µl/10ml) in 50mM carbonate-bicarbonate buffer, pH 9.6. Coat polystyrene microtiter wells (NUNC Maxisorp) with 100µl of diluted mAb per well. Incubate overnight at 4°C.

2. Wash wells 3X with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30min at room temperature with 100µl 1% BSA-PBS-T. Wash 3x with PBS-T.

3. Add 100µl of diluted allergen standard, house dust or air filter samples. Incubate for 1 hour at room temperature.

3a. Make a control curve using doubling dilutions of the Universal Allergen Standard: The control curve dilutions are from 250-0.49ng/ml for Der p 1; from 100-0.2 ng/ml for Fel d 1; Pipette 20µl allergen standard into 180µl 1% BSA PBS-T into wells A1 and B1 of the ELISA plate. Mix well and transfer 100µl across the plate into 100µl 1% BSA PBS-T diluent to make 10 serial doubling dilutions. Wells A11, B11, and A12and B12 should contain only 1% BSA PBS-T as blanks.

3b. House dust samples are routinely diluted two-fold from 1/2 -1/80.

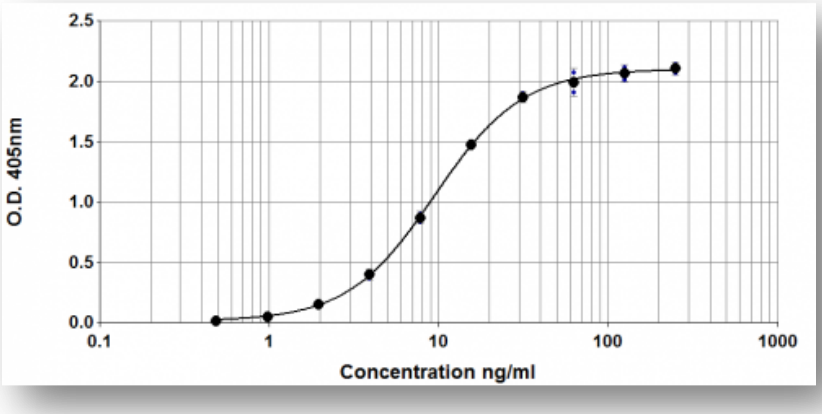
4. Wash wells 3x with PBS-T and add 100µl diluted biotinylated antibody (mAb 4C1 Der p1; mAb 3E4 Fel d1). The antibody solution contains 50% glycerol and should be diluted 1/1000 (i.e. 10µl/10ml) in 1% BSA-PBS-T. Incubate for 1 hour at room temperature.

5. Wash wells 3x with PBS-T and add 100µl diluted Streptavidin-Peroxidase (Sigma S5512, 0.25mg reconstituted in 1ml distilled water). The reconstituted Streptavidin should be diluted 1/1000 (i.e. 10µl/10ml) in1% BSA PBS-T. Incubate for 30 minutes at room temperature.

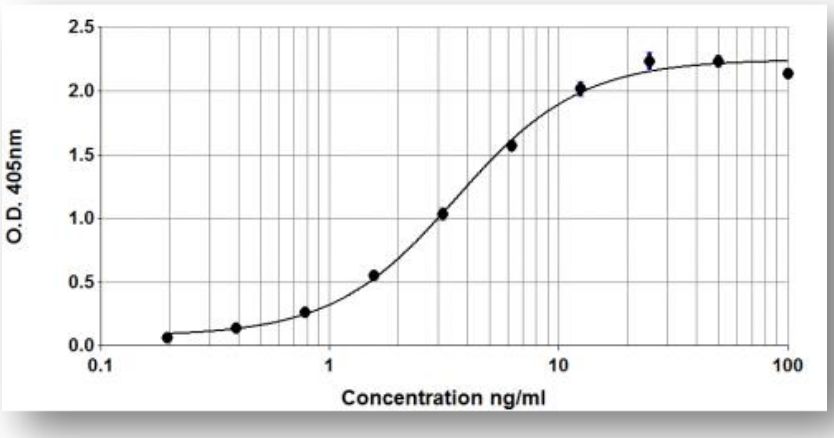
6. Wash wells 3x with PBS-T and develop the assays by adding 100µl ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

7. Read the plate when the optical density at 405nm reaches 2.0-2.4. (Fig. 2)

A



B



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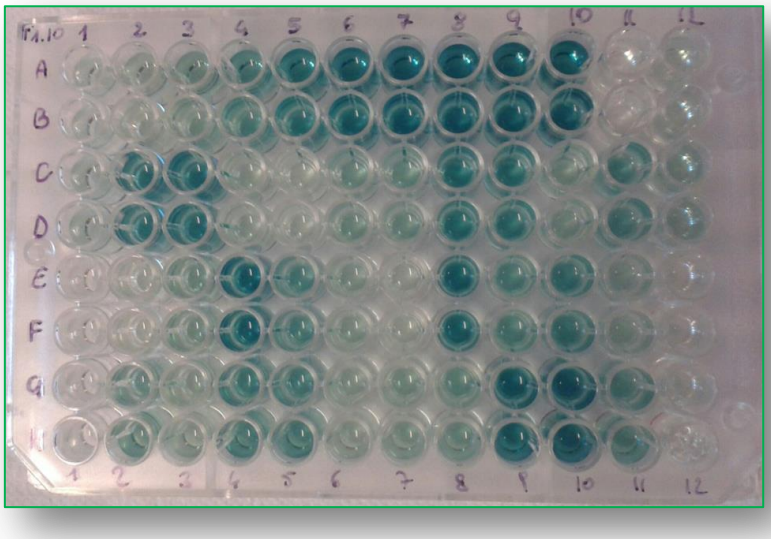

Fig.2 Der p1 **(A)** and Fel d1 **(B)** sample Curves (ELISA assay-Indoor Biotechnologies). Der p1 Elisa assays **(C)**: lines A and B from 1 to 10 are the Standard Curve, the wells 11 A and 11 B are the negative controls.

6. References

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Annex 1. Fieldwork booklet- General Information

General Information	
Country	
City	
School/House/office number	

School/House/office Coordinates	**name:**	
	street, number:	
	postal code:	
	City:	

Fieldworkers:	

General School/House/office Code	

Annex 2. Fieldwork booklet- Information on biological sampling

School/House/office IDcode	Room nr

BIOLOGICAL MEASUREMENTS

Type	Code	Date (dd-mm-YY)	Time (hr:min)	Sampling surface(1)	Vacuum duration(2) (min)	Vacuumed area(3) (m^2)	Remarks and/or Irregularities(4)
Filter 1(*)							
Filter 2(**)							

Notes:
(*) or "f1" describe the dustream filter used for the sampling before the entrance of pupils. (**) or "f2" describe the dustream filter used for the second sampling after the end of the lessons.
(1) describe what types of surfaces were vacuumed (eg. floors, carpets, desk, banks, windows, walls etc.)
(2) Describe the duration of the sampling (minutes).
(3) describe the approximate measure of sampling area.
(4) Note down any remarks or irregularities to the protocol that occurred