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Direct analysis of airborne mite allergen (*Der f*1) in the residential atmosphere by chemifluorescent immunoassay using bioaerosol sampler

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ABSTRACT

Dermatophagoides farinae allergen (*Der f*1) is one of the most important indoor allergens associated with allergic diseases in humans. Mite allergen *Der f*1 is usually associated with particles of high molecular weight; thus, *Der f*1 is generally present in settled dust. However, a small quantity of *Der f*1 can be aerosolized and become an airborne component. Until now, a reliable method of detecting airborne *Der f*1 has not been developed. The aim of this study was to develop a fiber-optic chemifluorescent immunoassay for the detection of airborne *Der f*1. In this method, the *Der f*1 concentration measured on the basis of the intensity of fluorescence amplified by an enzymatic reaction between the labeled enzyme by a detection antibody and a fluorescent substrate. The measured *Der f*1 concentration was in the range from 0.49 to 250 ng/ml and a similar range was found by enzyme-linked immunosorbent assay (ELISA). This method was proved to be highly sensitive to *Der f*1 compared with other airborne allergens. For the implementation of airborne allergen measurement in a residential environment, a bioaerosol sampler was constructed. The airborne allergen generated by a nebulizer was conveyed to a newly sampler we developed for collecting airborne *Der f*1. The sampler was composed of polymethyl methacrylate (PMMA) cells for gas/liquid phases and some porous membranes which were sandwiched in between the two phases. *Der f*1 in air was collected by the sampler and measured using the fiber-optic immunoassay system. The concentration of *Der f*1 in aerosolized standards was in the range from 0.125 to 2.0 mg/m^3 and the collection rate of the device was approximately 0.2%.

1. Introduction

Dermatophagoides farinae is a common house dust mite found in most homes in the world including Japan [1–7]. This house dust mite feeds on dead skin and dander, so it is often found in places occupied by people and animals [8–10]. For example, it thrives in beds, carpets, furniture, and clothing owing to the abundance of human dander in such places. More importantly, *D. farinae* produces several allergens, among which *Der f*1 is the major allergen produced by this mite and is associated with asthma, eczema and allergic rhinitis in humans [11,12]. *Der f*1 is a cysteine protease, which is a digestive enzyme in the intestines of mites [13–15]. *Der f*1 is mainly associated with mite feces, and the majority of mite allergens have been found in settled dust. However, a small portion of reservoir dust can be aerosolized, resulting in an airborne *Der f*1 load [16]. To treat patients with allergic diseases, there are several approaches such as aeroallergen avoidance, medication to control symptoms, and immunotherapy of allergy. Among these approaches, the avoidance of exposure to indoor allergens is the most important measure for the prevention of atopic and allergic individuals. Therefore, a simple and quantitative monitoring technique is needed to determine airborne allergen levels, particularly in the homes of individuals with allergic diseases including asthma. Thus, the development of an airborne allergen sampling technique and allergen detection by a sensitive method are among the important requirements of on-site airborne allergen measurement.

For the detection of *Der f*1, several techniques have already been reported. In particular, immunological assays such as enzyme-linked

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immunosorbent assay (ELISA) [17], radio immunoassay (RIA), and electron spin resonance (ESR) radical immunoassay [18] have been reported to be quantitative and highly sensitive methods. However, they require the use of large detectors, which are difficult to use in a house and they are difficult to develop a continuous immunoassay system by integration with other technical components. Therefore, in recent years, fiber-optic biosensors have been widely examined. These biosensors require the use of a combination of biological receptors and physical or chemical transducers, which represent a new technology, and they show high sensitivity, require a low sample volume, and enable fast detection in the measurement of absorbance, fluorescence, and scattering characteristics [19–23]. Although we have already constructed a fiber-optic immunoassay system for *Der f*1 using general-purpose equipment [24], this system is difficult to integrate with other elements for the monitoring of airborne allergens.

On the other hand, for airborne particle sampling, several techniques are already available [25–27]. In particular, there are commercial devices for the transfer of airborne particles directly into a liquid sample based on the impinger sampling method or cyclonic sampling method [28,29]. For laboratory-scale experiments, an electrostatic sampler using an electrostatic field to collect a charged bioaerosol has also been developed as an aerosol-to-hydrosol sampling technique [30]. However, because these techniques require a high impact force or high voltage, it is difficult to use them as airborne *Der f*1 samplers in a house and to integrate them with the immunoassay system for the monitoring of airborne allergens.

In this study, we have constructed a fiber-optic chemifluorescent immunoassay system and a new airborne allergen sampler (bioaerosol sampler) for the analysis of airborne *Der f*1 in a residential environment. After the construction of the immunoassay system and the evaluation its characteristics, the airborne allergen sampler was constructed and applied to detect *Der f*1 in real house dust. The purpose of this study was to evaluate an integrated system for sampling and monitoring airborne allergen *Der f*1, which is present in many homes infested by *D. farinae* worldwide.

2. Materials and methods

2.1. Setup for fiber-optic chemifluorescent immunoassay

The measurement principle of the fiber-optic immunoassay system is based on a chemifluorescent assay. We used 10-acetyl-3,7-dihydroxyphenoxazine (ADHP) as a substrate [31,32]. ADHP is a nonfluorescent compound that reacts with horseradish peroxidase (HRP) according to the equation presented below [32]. It produces resorufin, a soluble, highly fluorescent reaction product with excitation/emission maxima of ~570/585 nm.

$$\text{ADHP (nonfluorescent)} + H_2O_2 \xrightarrow{\text{HRP}} \text{resorufin (fluorescent)}$$

The measurement system (Fig. 1) consisted of a green light-emitting diode (LED) excitation system, a photomultiplier tube (PMT), a bifurcated optical fiber assembly, filter holders, and an optical fiber probe. The LED (λ_p, 520 nm; OSPG5111P, OptoSupply, China) was driven by a DC power source (P4L, Matsusada Precision, Japan) with a current of 20 mA. Two band-pass filters (BPF) were used in the system. One transmitted wavelength of 570 ± 10 nm (MX0570, Asahi Spectra, Japan) on the excitation light side, the other transmitted wavelength of 600 ± 10 nm (MC600, Asahi Spectra, Japan) on the fluorescence side. A Y-shaped bifurcated optical fiber assembly (BIF600-VIS/NIR, Ocean Optics, USA) had two fibers of the same diameter (φ: 600 μm) side-by-side at the common end. One of the branched terminals of the assembly was connected to the filter holder, which transmitted excitation light. The other end was connected to the other filter holder to transmit the fluorescence of resorufin. The two-in-one end of the assembly was connected to an optical fiber probe made of plastic optical fiber (φ, 1 mm; core, polystyrene; cladding, polymethyl methacrylate (PMMA), Shenzhen Corpereal Photoelectric, China). The plastic fiber was cut into 12 cm, and both the incisal end faces were horizontally polished using a fiber-optic micropolisher (Rev™, Krell Technologies, USA), which is used for polishing films in the order corresponding to roughness of 30 μm for silica film, 9 μm for silica film, and 0.3 μm for alumina film. The fluorescent signals of resorufin were guided to the PMT modules (H7421, Hamamatsu Photonics, Japan). The imperceptible fluorescent light was collected by the PMT, amplified, and digitized using a PC.

2.2. Fiber-optic chemifluorescent immunoassay protocol and reagents

First, a capture antibody (INDOOR Biotechnologies, USA) diluted with 80 mmol/l carbonate–bicarbonate buffer (2 μg/ml) was added to microtiter plate wells (polystyrene microplate, 96 wells, Greiner, Germany) and incubated overnight (4 °C) to immobilize the antibody. Unbound antibodies were removed by washing the plate with 10 mmol/l phosphate buffer of pH 7.4 (PB)+0.05% Tween 20 (PBT). Blocking reagent, which was 1% bovine serum albumin (BSA, Itoham Foods, Japan) in PB was added and incubated (1 h, room temperature (RT)). After washing the plate, *Der f*1 standards (0.49–250 ng/ml diluted PBT, INDOOR Biotechnologies, USA) were then added and incubated (2 h, RT) with the solid-phase antibodies. After washing away unbound *Der f*1, biotin-conjugated detection antibodies (2 μg/ml, INDOOR Biotechnologies, USA) were added (1 h, RT). By binding these detection antibodies to *Der f*1, sandwiched immune complexes were obtained. Following washing to remove unbound detection antibodies, the detection reagent of streptavidin–peroxidase (streptavidin–HRP, 0.25 μg/ml; S5512, Sigma-Aldrich, USA) was added (0.5 h, RT). The plate was washed, a substrate solution of ADHP (QuantaRed Enhanced Chemifluorescent HRP Substrate Kit, Thermo Fisher Scientific, USA) was added and a resorufin fluorescence was developed. All reagents were added in the volumes of 100 μl. After a 3 min incubation, the stop solution (10 μl) in the kit was added to stop the enzymatic reaction. The microtiter well was placed in the fiber-optic immunoassay system, and the fluorescence signal was recorded using the PC connected to the PMT. The exposure time was fixed at 1.0 s in all experiments.

To determine selectivity to other airborne allergens, the antigen solutions measured were not only solutions of *Der f*1 but also those of other allergens or a mixture of *Der f*1 and another allergen. The allergens used for the investigation were *Der f*2 (SEIKAGAKU Corp., Japan), *Der p*1 (ITEA, Japan), *Cry j*1 (Hayashibara Biochemical Labs, Japan), *Amb a*1 (ITEA, Japan), *Alt a*1 (ITEA, Japan) and *Can f*1 (ITEA, Japan).

2.3. ELISA protocol

ELISA is one of a standard method for the quantification of dustborne *Der f*1. *Der f*1 ELISA was performed in accordance with the manufacturer's recommended protocol (INDOOR Biotechnologies, USA). Each experiment was carried out in triplicate. The ELISA protocol from the immobilization of capture antibodies to the incubation with streptavidin–HRP was the same as that described in Section 2.2. After that, the plate was washed and a substrate solution (1 mmol/l 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonate acid) diammonium salt (ABTS) in 70 mmol/l citrate phosphate buffer; ABTS, A1888, Sigma-Aldrich, USA) was added. The color was developed in proportion to the amount of bound *Der f*1. The absorbance was measured at 450 nm using a microplate reader (SH-1000Lab, CORONA Electric, Japan).

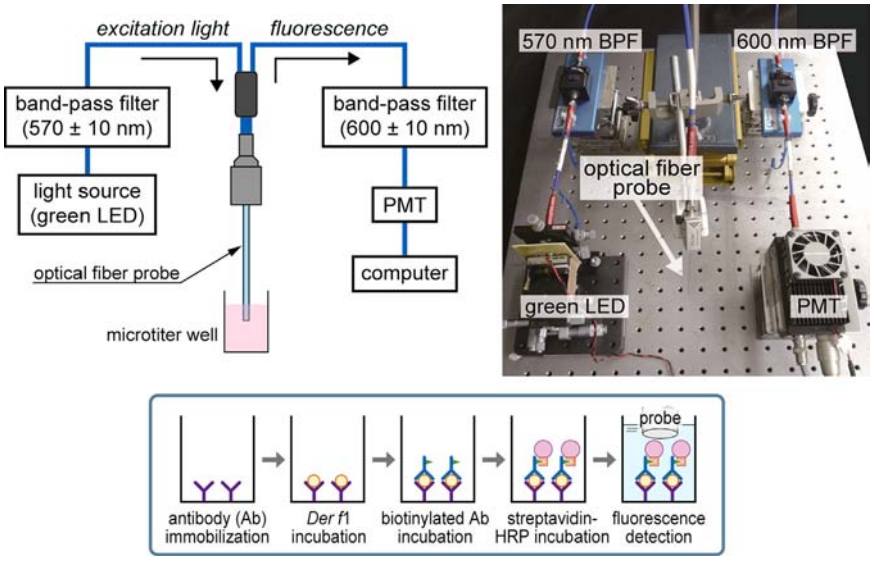

Fig. 1. Experimental setup of the fiber-optic chemifluorescent immunoassay system and principle of *Der f*1 detection. The excitation light source (LED) is coupled to the optical fiber probe, and the fluorescence of resorufin produced by the antigen–antibody complex is detected coaxially by the photomultiplier tube.

2.4. Experimental setup and procedure for sampling of airborne *Der f1*

The airborne allergen sampling system is shown in Fig. 2(a). The system is composed from two parts, one for the generation of the airborne allergen and the other for sampling. Airborne *Der f*1 particles were produced using a nebulizer (particle φ: approximately 5 μm, 400 μl/min, NE-C28, OMRON Healthcare, Japan). Five *Der f*1 solutions (10.0, 7.5, 5.0, 2.5 and 0.125 μg/ml; INDOOR Biotechnologies, USA) were used for the production of airborne particles, which were sprayed in to the conductive resin chamber (1000 ml, SANPLATEC, Japan) for 2 min. The chamber was connected to an air-conveying system (FK-6, BLS, Japan) via a plastic (polyvinyl chloride) tube. The air-conveying system generates a suction/discharge flow by means of compressed air. The conveying flow rate was consistently maintained at 2 L/min by regulating the compressed air pressure. In allergen sampling, the air-conveying system was turned on for 2 min to convey airborne *Der f*1 to the originally designed bioaerosol sampler. Fig. 2(b) shows the constructed sampler. The device was fabricated using porous membranes mounted between two PMMA cells intended for a two-phase cell (gas/liquid). For the gas phase, through-holes, whose diameter and height on one side were 19 mm and 5 mm, and on the other side were 13 mm and 8 mm, respectively, were created in a PMMA block of 50 mm × 50 mm × 13 mm (width × length × height). For the liquid phase, half of a commercial dialysis cell (FA-1, SANPLATEC, Japan) was used. The cavity volume capacity of the cell was 1.5 ml, the hole dimensions ($\varphi \times H$ mm) were 13 × 5, and the PMMA cell dimensions ($W \times L \times H$ mm) were 50 × 50 × 13. The other components of the sampler were as follows (in order from the liquid phase): a tetrafluoroethylene-perfluoroalkylvinylether copolymer (PFA) net (F-3220-01, Flon Industry, Japan), an aqueous cellulose membrane (M-3II, Asahi Kasei Fibers, Japan), an ethylene-tetrafluoroethylene copolymer (ETFE) mesh (F-3006-9A-1800, Flon Industry, Japan), a silicone sheet (0.5-mm-thick, WEB7020, SANPLATEC, Japan), a PMMA block of 50 × 50 × 3 ($W \times L \times H$) with a hole (φ: 13 mm), double stainless washers (outer diameter, 12 mm; inner diameter, 5 mm; thickness, 0.8 mm). The airborne *Der f*1 from the air-conveying system was taken into the liquid phase filled with 1.5 ml PB through the porous membranes. After the uptake of the airborne *Der f*1, the aqueous solution was obtained as the allergen specimen from the liquid phase, and the *Der f*1 level was measured by fiber-optic chemifluorescent immunoassay.

In addition to the sampling of the airborne *Der f*1 from the purified allergen solution, *Der f*1 in actual dust samples was detected using the airborne allergen sampling system. Dust samples were prepared as reported previously [23]. Each of 5 mg dust that was commercially available house dust (freeze-dried dust, D9, GREER®, USA) and four different dust samples separately collected from carpet, mattress, pillow and blanket were conveyed to the allergen sampling device, and allergen specimens were obtained.

3. Results and discussion

3.1. Measurement of standard Der f1 level

The calibration curves for standard *Der f*1 levels measured by chemifluorescent immunoassay and ELISA are shown in Fig. 3. The solid line in Fig. 3 shows the calibration curve obtained by plotting the mean fluorescence intensity for each standard level measured by the PMT in the fiber-optic chemifluorescent immunoassay system. The calibration curve of the ELISA results is also shown in Fig. 3 as a dashed line. The error bar of each plot represents the standard deviation of five measurements. The sigmoid curves were fitted by nonlinear regression analysis. The correlation coefficient calculated from the curves (Fig. 3) for both immunoassays was 0.999. The equations were as follows.

$$\text{intensity (cps)} = -45577 + 52005/(1+e^{(-([Derf1(ng/ml)]+14.39)/7.45)})$$

$$\text{absorbance (arb.unit)} = -1.56 + 2.49/(1+e^{(-([Derf1(ng/ml)]+4.80)/9.37)}) \quad (1)$$

The lowest allergen concentration that gave a positive signal compared with a control (no *Der f*1) was 0.49 ng/ml; therefore, this value was considered as the lower limit of detection for both methods. The upper limits for chemifluorescent immunoassay and ELISA were 250 ng/ml and 125 ng/ml, respectively. Therefore, the immunoassay system was sufficiently quantitative and its dynamic range was suitable for use in *Der f*1 measurement. The precision (reproducibility) of chemifluorescent immunoassay was examined

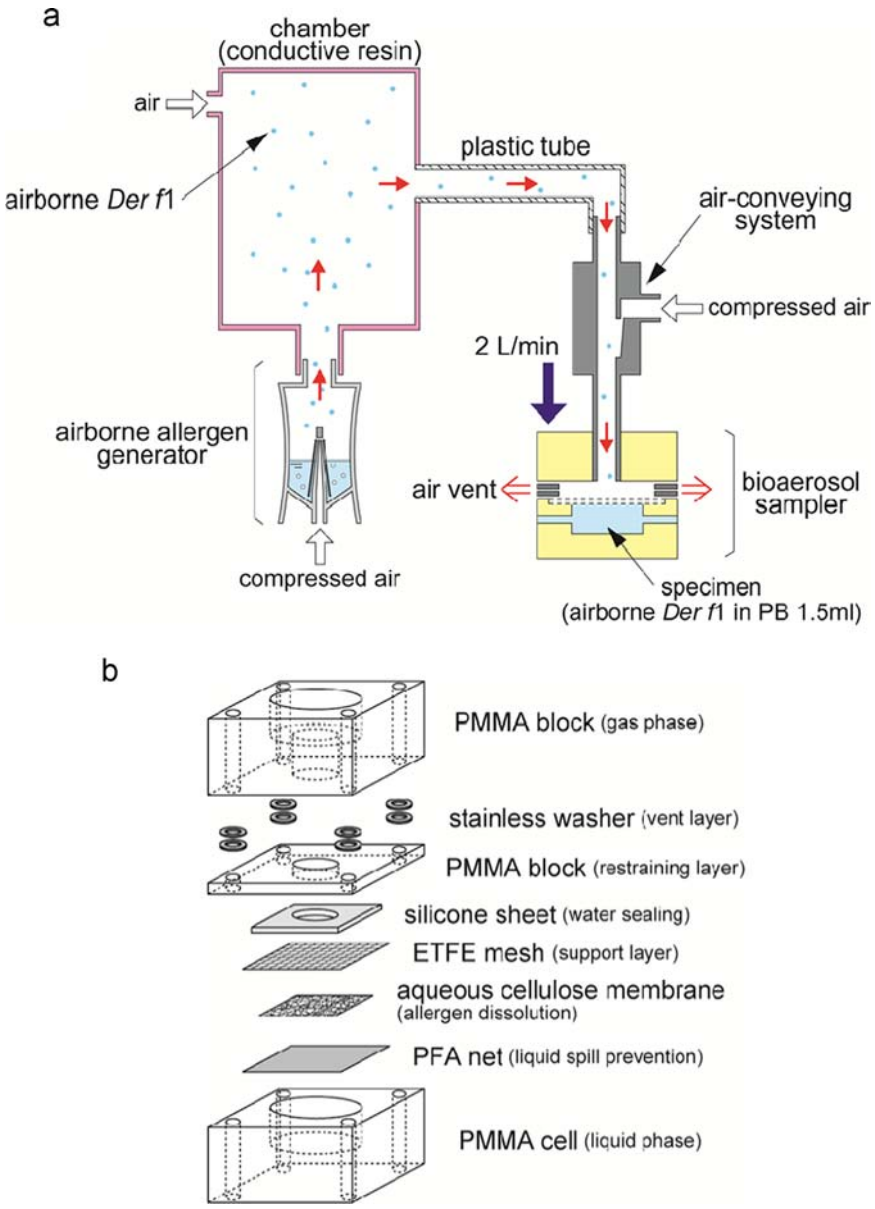

Fig. 2. Experimental setup for sampling of airborne allergen. (a) Entire setup for allergen sampling. Airborne allergens generated by the nebulizer are collected into the chamber and conveyed to the bioaerosol sampler. (b) Structural detail of the bioaerosol sampler. Airborne allergens are collected from the gas phase to the liquid phase via porous membranes.

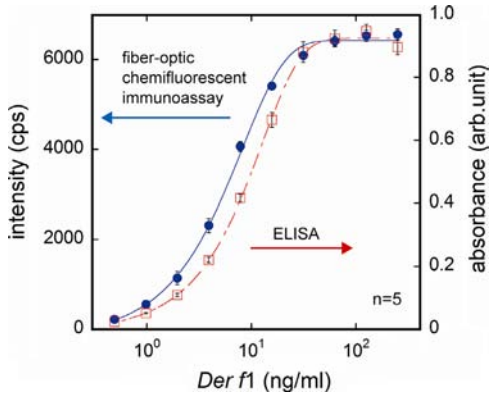

Fig. 3. Calibration curves for *Der f*1 measured by the chemifluorescent immunoassay system and ELISA. The detection limit and calibration range of the system are similar those of ELISA, which is conventionally used for *Der f*1 measurement.

using one sample (15.63 ng/ml, $n=5$). The coefficient of variation was 1.31%.

3.2. Selectivity of the system for Der f1 detection

To evaluate the selectivity for these assays and to examine the cross-reactivity of *Der f*1 with other allergens, nine additional assays were performed and several indoor allergens were included in the assays. The following indoor allergens were used: *Der f*2, a house dust mite (*D. farinae*) allergen; *Der p*1, a house mite allergen (*Dermatophagoides pteronyssinus*); *Can f*1, a dog dander allergen (*Canis familiaris*); *Cry j*1, a cedar (*Cryptomeria japonica*) pollen allergen; *Amb a*1, a ragweed pollen allergen; *Alt a*1, an allergen of a fungal species (*Alternaria alternata*), and mixtures of *Der f*1 + *Der f*2 and *Der f*1 + *Der p*1. The concentrations of these allergens were all 10 ng/ml. These allergens are typical household allergens. The allergen solutions were measured using the immunoassay system.

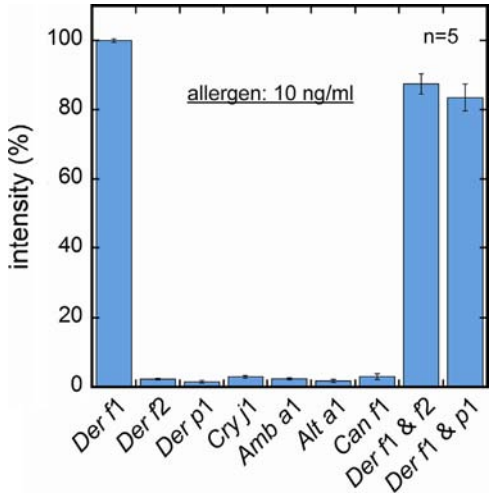

Fig. 4. Selectivity of the chemifluorescent immunoassay system to other airborne allergens. Positive signals were observed only for *Der f*1.

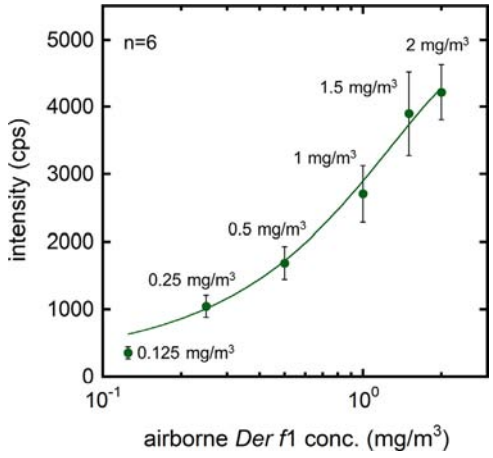

Fig. 5. Comparison of fluorescence intensities based on the difference in the concentration of airborne *Der f*1. Positive signals are obtained in the range from 0.125 to 2 mg/m^3.

Fig. 4 shows the selectivity of the *Der f*1 chemifluorescent immunoassay. The fluorescence intensity of signals from a *Der f*1 sample was defined as 100%. The fluorescence intensity of signals from other allergens was low ($<4\%$). These results showed that the chemifluorescent immunoassay is a highly specific method for the detection of *Der f*1. The reason for the slightly drop in the fluorescence intensity of signals from mixture samples can be explained by the fact that the capture antibody have little cross-reactivity with other allergens. In particular, *Der f*1 and *Der p*1 are Group 1 mite allergens, which are cysteine proteases and share 81% sequence identity [33–35]. A common epitope present on both *Der f*1 and *Der p*1 has also been identified [36]. Therefore, it is expected that *Der p*1 competitively blocks the binding of *Der f*1 to the capture antibody. Because the effect of competitive inhibition was negligibly small, the fiber-optic chemifluorescent immunoassay system was applied to measure actual house dust samples in the following experiment.

3.3. Measurement of airborne Der f1

For the on-site measurement of airborne allergens, the airborne allergen sampling system consisting of the newly developed bioaerosol sampler was constructed and evaluated. As an airborne generator, a commercial nebulizer was used, which can generate a mist of particles with 5 μm diameter, which was within the definition of fine dust particles, that is, smaller than 10 μm. Fig. 5 shows the fluorescence intensity of the signals from the samples obtained from the bioaerosol sampler and measured by the fiber-optic immunoassay system. The fluorescence intensity of each airborne *Der f*1 specimen increased with the *Der f*1 concentration. Therefore, the measurement of airborne allergens was successfully accomplished using the bioaerosol sampler and the fiber-optic immunoassay system. The relationship between the *Der f*1 concentration in air and fluorescence intensity was given by the following equation with the correlation coefficient of 0.994.

$$\text{intensity(cps)} = -7128.3 + 12281/(1+e^{(-([Der f1\ (\text{ng/ml})]+0.37)/0.92)})$$

Table 1 shows the collection rate of airborne *Der f*1 calculated using the measurement results of the specimens. In the sampling experiment, the flow rates of the nebulizer and sampling system were 400 μl/min and 2 L/min, respectively. Because the collection time was 2 min, 800 μl *Der f*1 solution was nebulized and 4 L air was conveyed to the sampler. The *Der f*1 concentrations of samples were calculated using Eq. (1). The amount of dissolved *Der f*1 in each sample was calculated using the concentration and volume (1.5 ml) of the sample. The collection rates of all the samples were approximately 0.2%.

Table 1
*Der f*1 volume collected by the sampler and collection rate of airborne allergen in the study.

*Der f*1 conc. in air (mg/m^3)	Total airborne *Der f*1* (μg)	Measured value of specimen		Collection rate (%)
		*Der f*1 conc. (ng/ml)	Collected *Der f*1 (ng)	
0.125	0.5	0.69	1.03	0.21
0.25	1.0	1.70	2.54	0.25
0.5	2.0	2.75	4.12	0.21
1.0	4.0	4.70	7.06	0.18
1.5	6.0	7.77	11.65	0.19
2.0	8.0	8.83	13.24	0.17
				ave. 0.20

* Estimated value of aerosolized *Der f*1 in 4 L air (flow rate, 2 L/min; collection time, 2 min).

According to the report of the World Health Organization (WHO) and Platts-Mills et al. [8,37], the guideline values of Group 1 allergens of *Dermatophagoides* sp. (*Der* 1) are as follows: a 2 μg/g dust should be regarded as posing a risk of sensitization, and a 10 μg/g dust should be regarded as a risk factor for acute attacks of asthma. On the other hand, the United States Environmental Protection Agency (EPA) set the guideline value of indoor particulate matter PM10 including fine dust at 150 μg/m^3 [38]. When the concentration of house dust is the same as that in EPA's guidelines, *Der* 1 guideline values for indoor air are translated as 0.3 μg/m^3 and 1.5 μg/m^3. Thus, it is preferable that the measurement method for airborne *Der f*1 has a detection limit much lower than these concentrations. Therefore, it is necessary to improve the sensitivity of our sampling system by at least three orders of magnitude. In particular, the ventilation layer of the sampling device was designed to decrease the air pressure. Therefore, a certain amount of airborne allergen in the sampling air was allowed to escape from the sampler without dissolving in the liquid phase. It is expected that reconsideration of the flow rate or the method of ventilating the sampling system may be one of the strategies to improve sensitivity.

To evaluate the applicability of the fiber-optic immunoassay system and bioaerosol sampling device for use in an actual living environment, house dust samples were collected and the *Der f*1

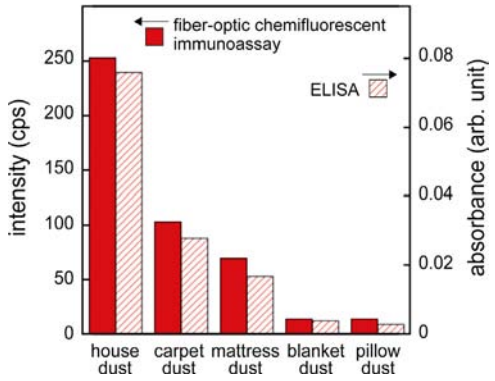

Fig. 6. Comparison between signals for *Der f*1 determined by chemifluorescent immunoassay system and ELISA.

level was measured using our measurement systems. House dust samples (5 mg) were collected from five different areas in a house using our bioaerosol sampler and measured by the fiber-optic chemifluorescent immunoassay system. Fig. 6 shows the fluorescence signal intensity and absorbance of the indoor specimens collected using our bioaerosol sampling device and detected by chemifluorescent immunoassay and ELISA, respectively. The results measured by two different methods showed a similar shift of signals and a high correlation (correlation coefficient of 0.998) according to the dust sampling area in the house. Therefore, the combination of the fiber-optic immunoassay system and the bioaerosol sampler is a promising technique for the determination of *Der f*1 concentration in actual house dust.

4. Conclusions

A fiber-optic chemifluorescent immunoassay system for the detection of airborne *Der f*1 was constructed. A plastic optical fiber was polished and used as a sensor probe in the system and a PMT was used as a detector of fluorescence. The limit of detection and concentration range of chemifluorescent immunoassay were similar to those of ELISA (0.49–250 ng/ml). Moreover, for the monitoring of airborne allergens on-site, a bioaerosol sampler was constructed and the *Der f*1 level was measured by the chemifluorescent immunoassay system. The sampler was composed of PMMA cells for the gas/liquid phases and some porous membranes. The concentration range of *Der f*1 in the air samples was from 0.125 to 2.0 mg/m^3, and the trapping rate of the device was approximately 0.2%. In addition, airborne *Der f*1 was determined in a residential environment by chemifluorescent immunoassay and ELISA. In the future, the integration of the immunoassay system and the sampler will be one of the basic techniques for the airborne allergen monitoring in a living environment.

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