Environ. Sci. Technol. **2009,** *43,* 3233–3239

Rapid Detection and Quantification of *Aspergillus fumigatus* in Environmental Air Samples Using Solid-Phase Cytometry

LIES M. E. VANHEE, HANS J. NELIS, AND TOM COENYE*

Laboratory of Pharmaceutical Microbiology, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium

Received December 3, 2008. Revised manuscript received March 6, 2009. Accepted March 9, 2009.

Aspergillus fumigatus is an ubiquitous fungus capable of causing severe infections such as aspergilloma, allergic bronchopulmonary aspergillosis, and invasive aspergillosis, especially in immunocompromised patients. Monitoring the number of *Aspergillus fumigatus* spores in the air is crucial for infection control. In the present study, a novel approach for the quantification of *Aspergillus fumigatus*, based on solid-phase cytometry (SPC) and immunofluorescent labeling, was developed. The sensitivity and specificity of the assay were confirmed by testing pure cultures. *Paecilomyces variotii* and *Rhizopus stolonifer* were codetected but could be excluded on the basis of morphology of the microcolonies. The SPC method has considerable advantages compared to the culture-based method, including its low detection limit (4 cells/m^3), its speed (results are obtained within 24 h), and the straightforward microscopic identification of *Aspergillus fumigatus*. Additionally, comparison of results obtained with both methods demonstrated that they are equally accurate for the quantification of *Aspergillus fumigatus* in environmental air samples.

1. Introduction

In recent years, concerns of human exposure to microorganisms originating from air have been centered on fungi (*1, 2*). In particular, immunocompromised patients are susceptible to allergic, infectious and toxic reactions caused by airborne fungi (*1, 3*). *Aspergillus fumigatus* is a common environmental fungus, capable of causing several respiratory diseases such as allergic bronchopulmonary aspergillosis and aspergilloma. However, it can also lead to the development of invasive disease in immunocompromised patients (*4*−*6*). Invasive aspergillosis (IA) has a very high mortality rate, ranging from 50 to 100% (*5, 7, 8*). Because of its difficult diagnosis and subsequent treatment, prevention plays a key role in the infection control of IA (*5*−*8*).

Genotyping studies have revealed that indoor hospital air is often the source of *Aspergillus* infection (*9, 10*). However, only in a few studies was this link confirmed (*4, 11*−*13*). Additionally, no acceptance level can be determined because of the difference in infectious concentrations (1−10 cells/m^3) found depending on the sampling and analysis method (*12, 13*).

Accurate methods to determine the level of airborne *A. fumigatus* are required to evaluate the effect of construction work, laminar airflow and high-efficiency particulate air filtration in hospitals (*14*−*17*) but also to study the link between *A. fumigatus* and sick building syndrome (*18*−*20*) and to monitor the exposure in wood chipping facilities (*21*) and near compost heaps and hay barns (*12*). Therefore, a new quantification method must have both a low lower and a high upper limit of quantification (LLQ and ULQ, respectively) (*17, 21*).

The traditional method for quantification of *A. fumigatus* in air samples relies on culture and subsequent identification using microscopy. This method has several limitations such as the need for a long (minimum 4 days) incubation period and the difficulty in choosing a medium that combines selectivity with a maximum recovery of *A. fumigatus*. Moreover, accurate microscopic identification of *A. fumigatus* requires a high level of expertise and is time-consuming (*2, 16, 22*−*24*).

Several alternatives with improved specificity and speed compared to culture and microscopy have been proposed for the specific detection of *A. fumigatus* in air samples. For example, an assay based on concentration measurement of the allergen Asp f 1 and a quantitative polymerase chain reaction (qPCR) method have been developed. However, the correlation between the amount of Asp f 1 antigen or *A. fumigatus* DNA and the number of cells is often questioned and nonviable cells are also determined (*20, 21, 25, 26*).

Recently, we have developed a novel approach for the rapid enumeration of airborne bacteria and fungi based on solid-phase cytometry (SPC) (*27*). Air samples are collected by impaction on a water-soluble polymer that is subsequently dissolved. Finally, the traditional steps of SPC including filtration, fluorescent labeling, scanning and microscopic confirmation are performed (*28, 29*). In SPC, the combination of the automatic detection of the fluorescent spots and the microscopical validation results in an easy and accurate technique for quantification (*30, 31*). Other important advantages of SPC are its speed and low detection limit (one cell per filtered volume) (*28, 29*).

In the present paper, a method based on SPC and immunofluorescence for the rapid and specific detection of *A. fumigatus* in air samples is described. Results obtained with SPC for artificially contaminated and environmental air samples were compared to results obtained with a culture-based method.

2. Materials and Methods

2.1. Development of SPC Method. *2.1.1. Preparation of the Water-Soluble Polymer.* A 10% (w/v) polyvinylalcohol solution (PVA, Sigma, St. Louis, MO) was filtered through a cellulose acetate membrane filter with a pore size of 0.22 μm (Corning Inc., Glendale, AZ). Petri dishes containing 15 mL of the PVA solution were left open in a vertical LAF cabinet for 11 h to obtain dry polymer films.

2.1.2. Air Sampling. For the collection of airborne *A. fumigatus*, the MAS-100 *Eco* impaction sampler operating at a flow rate of 100 L/min (Merck, Whitehouse Station, NJ) was used (*32*). Air was impacted on a water-soluble polymer for subsequent SPC analysis. The MAS-100 *Eco* air sampler was selected on the basis of its excellent capturing performance in previous experiments (*33*).

2.1.3. Filtration and Microcolony Formation. Using sterile tweezers, the polymer films were removed from the Petri dish immediately after sampling and dissolved in 20 mL of 0.9% (w/v) NaCl. Subsequently, 5 mL of the solution was

* Corresponding author phone: (32) 9 264 8141; fax: (32) 9 264 8195; e-mail: Tom.Coenye@UGent.be.

10.1021/es803435a CCC: $40.75 © 2009 American Chemical Society
Published on Web 03/27/2009

TABLE 1. Results Obtained with SPC and Culture for Air Samples Artificially Contaminated with *A. fumigatus*[a]

fungus[b]	*A. fumigatus* cells/m^3 (average ± SE) ($n = 3$) SPC	culture
A. fumigatus IHEM 2494	1867 ± 698	1853 ± 713
A. fumigatus IHEM 2952	950 ± 683	223 ± 97
A. fumigatus IHEM 3007	8317 ± 1040	>ULQ[c]
A. fumigatus IHEM 3768	417 ± 117	833 ± 44
A. fumigatus IHEM 4184	3317 ± 934	2853 ± 71
A. fumigatus IHEM 4185	200 ± 150	843 ± 188
A. fumigatus IHEM 4187	20433 ± 4297	>ULQ[c]
A. fumigatus IHEM 4189	10017 ± 2197	>ULQ[c]
A. fumigatus MUCL 978	2500 ± 898	2850 ± 363
A. fumigatus MUCL 15821	3750 ± 776	2357 ± 364
A. fumigatus CI 1	850 ± 400	693 ± 180
A. fumigatus CI 2	3267 ± 721	>ULQ[c]
A. fumigatus CI 3	1025 ± 551	507 ± 22
A. fumigatus CI 4	850 ± 493	563 ± 93
A. fumigatus CI 5	8383 ± 3606	>ULQ[c]
A. fumigatus CI 6	7717 ± 5374	2160 ± 924
A. fumigatus CI 7	567 ± 309	170 ± 87
A. fumigatus CI 8	575 ± 265	323 ± 43
A. fumigatus CI 9	1550 ± 350	1387 ± 134
A. fumigatus CI 10	283 ± 209	313 ± 45
A. fumigatus CI 11	1017 ± 643	200 ± 0
A. fumigatus CI 12	3250 ± 2701	1970 ± 1022
A. fumigatus CI 13	10100 ± 407	>ULQ[c]
A. fumigatus CI 14	3450 ± 2694	>ULQ[c]
A. fumigatus CI 16	20683 ± 3804	>ULQ[c]

[a] No statistically significant differences between SPC and culture (Mann-Whitney test, $p < 0.01$) were found. [b] IHEM, Instituut voor Hygiene en Epidemiologie, Brussels, Belgium; MUCL, Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; CI, clinical isolate from our own collection. [c] The upper limit of quantification (ULQ) of the culture-based method was reached.

TABLE 2. Results Obtained with SPC and Culture for Air Samples Collected at 23 Various Locations[a]

location	*A. fumigatus* cells/m^3 (average ± SE) ($n = 3$) SPC	culture
outdoor environment	<LLQ[b]	4 ± 2
office	<LLQ[b]	ND[c]
student room	<LLQ[b]	1 ± 1
basement	5 ± 3	3 ± 1
bathroom	12 ± 2	8 ± 0
shower 1	23 ± 9	18 ± 2
shower 2	<LLQ[b]	7 ± 2
refrigerator	<LLQ[b]	3 ± 2
laboratory 1	<LLQ[b]	8 ± 0
laboratory 2	<LLQ[b]	5 ± 2
laboratory 3	<LLQ[b]	ND[c]
laboratory 4	<LLQ[b]	ND[c]
botanical garden 1	23 ± 11	9 ± 2
botanical garden 2	17 ± 9	NI[d]
botanical garden 3	<LLQ[b]	10 ± 7
false ceiling 1	<LLQ[b]	7 ± 2
false ceiling 2	38 ± 38	7 ± 1
ship 1	<LLQ[b]	NI[d]
ship 2	<LLQ[b]	NI[d]
attic after renovation	57 ± 38	NI[d]
attic next to renovation site	22 ± 14	NI[d]
construction site 1	<LLQ[b]	6 ± 6
construction site 2	8 ± 6	2 ± 2

[a] No statistically significant differences between SPC and culture (Mann-Whitney test, $p < 0.01$) were found. [b] The lower limit of quantification (LLQ) of the SPC procedure was reached for minimum one replicate of these samples. [c] ND, not detected; no *A. fumigatus* colonies were found using the culture-based method. [d] NI, not interpretable; culture plates were completely overgrown with a fungus different from *A. fumigatus*, making interpretation impossible.

filtered through a black polycarbonate membrane filter with a 0.4 μm pore size (Millipore, Bedford, MA). Afterward, the filter was transferred to a cellulose pad soaked with 600 μL of growth medium. This growth medium was prepared by supplementing Sabouraud glucose broth (Oxoid, Hampshire, U.K.) with an antibacterial mixture containing ticarcillin (4688 μg/L medium) and clavulanic acid (312 μg/L) (Timentin) (Smithkline Beecham Pharma, Genval, Belgium). Additionally, a mixture of growth factors containing pyridoxine (10 μg/mL), thiamine (1.0 μg/mL), nicotinic acid (0.1 μg/mL), riboflavin (10 μg/mL), panthothenate (1.0 μg/mL), *para*-aminobenzoic acid (10 μg/mL), and inositol (10 μg/mL) (Sigma) was also added to the medium. The filter and cellulose pad were incubated in an Analyslide (Gelman Sciences, Ann Arbor, MI) at 47 °C for 18 h.

2.1.4. Labeling and Counterstaining. Filters were placed on top of 100 μL of a blocking buffer (Invitrogen, Carlsbad, CA) for 30 min at 30 °C. After blocking, the filter was air-dried for a few seconds. Subsequently, *A. fumigatus* microcolonies were detected by placing the filter on top of 100 μL of a 2 μg/ml solution of a monoclonal mouse anti-*Aspergillus* antibody (343/31 Clone, AbD Serotec, Oxford, U.K.) in blocking buffer and incubated at 30 °C for 30 min. Afterward, tyramide signal amplification (TSA) was used to fluorescently label the microcolonies. Therefore, the filter was placed on top of 100 μL of secondary HRP-conjugated goat antimouse antibody (Invitrogen) solution (10 μg/mL blocking buffer) for 30 min at 30 °C. Finally, the filter was transferred onto 100 μL of Alexa Fluor-tyramide (Invitrogen) solution (stock solution diluted 1:50 in amplification buffer according to the manufacturer's instructions) and incubated in the dark at 25 °C for 30 min. Between different steps, filters were rinsed three times with 1 mL of phosphate buffered saline (0.14 M NaCl, 3 mM KCl, 3 mM $Na_2HPO_4 \cdot 12H_2O$, 1.5 mM KH_2PO_4) by filtration. Following the final labeling step, interfering particles were counterstained with 1 mL CSE/2 (AES-Chemunex, Ivry-sur-Seine, France) (*34*). All reagents used in the SPC protocol were filtered (filter pore size 0.22 μm) before use.

2.1.5. Scanning. The filters with labeled microorganisms were placed on a holder, on top of a nitrocellulose membrane (Millipore) moistened with 100 μL of Citifluor AF3 (Citifluor, Leicester, U.K.) and scanned by the ChemScan *RDI* (AES-Chemunex). This solid-phase cytometer consists of an argon laser emitting light of 488 nm and two photomultiplier tubes that detect the fluorescent light emitted by the labeled cells. The produced signals are processed by a PC, applying a series of software discriminants to differentiate valid signals associated with labeled *A. fumigatus* microcolonies from abiotic, fluorescent particles. Subsequently, results are displayed as green spots on a membrane filter image in a primary and, after software elimination of background, secondary scan map (*29*).

Validation. To confirm the results, visual inspection using an Olympus BX40 epifluorescence microscope (Olympus, Tokyo, Japan) was performed. The filter holder was placed on a computer-driven moving stage in exactly the same position as in the Chemscan *RDI*. Subsequent highlighting of a green spot in the secondary scan map on the PC resulted in the direction of the microscope to the respective position on the membrane filter (*28, 30*), which allowed rapid visual inspection of all fluorescent spots.

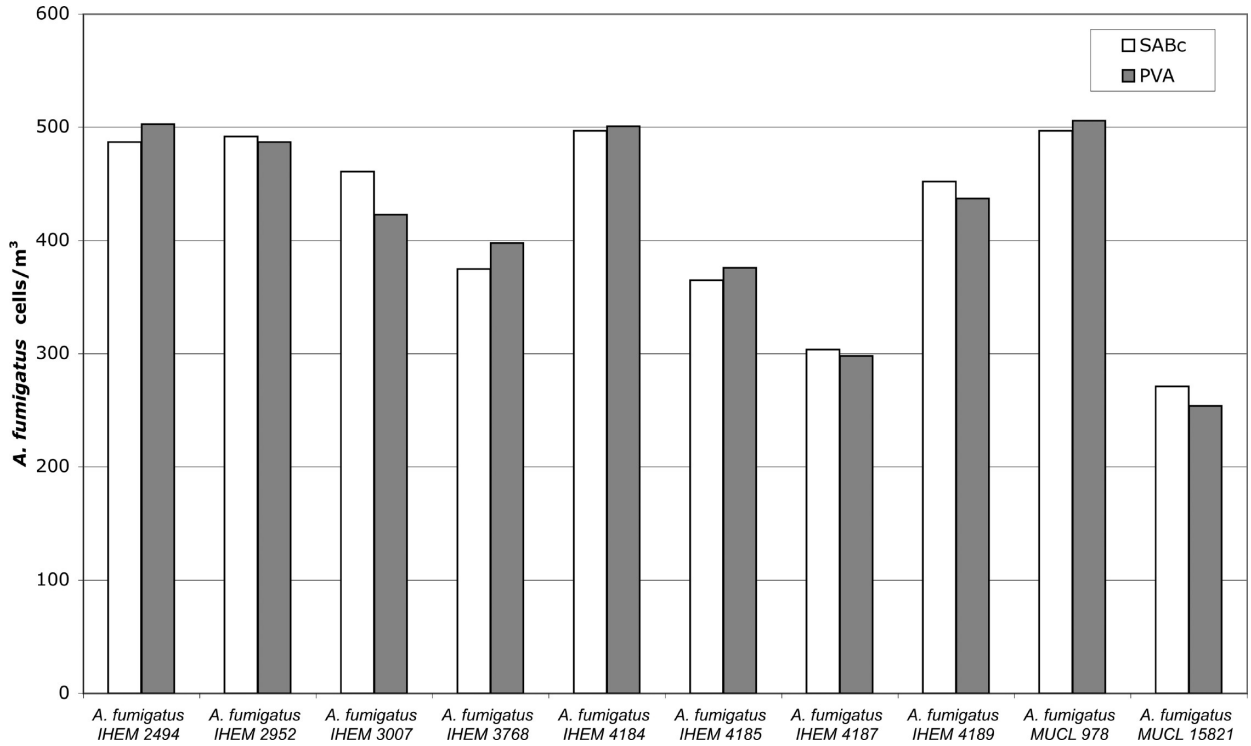


FIGURE 1. Results obtained by culturing air samples collected on PVA and SABc. To that end, the air was artificially contaminated with 10 different *A. fumigatus* strains.

2.2. Evaluation of the Capturing Efficiency of the PVA Film. To check whether impaction on the PVA polymer and impaction on a culture plate are comparable, we collected air samples in triplicate on both media. To that end, air was artificially contaminated with ten different *A. fumigatus* strains (see 2.5.) and 50 L samples were collected. The PVA solution was filtered and subsequentely the filter was placed on the same culture medium (Sabouraud agar (Oxoid) supplemented with 0.1% chloramphenicol (Sigma), SABc). Both plates were incubated at 47 °C and the number of colonies was determined.

2.3. Determination of the Lower Limit of Quantification and Evaluation of the Linearity of the Assay. As the theoretical detection limit of SPC is one cell per filter and only one-fourth of the air sample is filtered (5 mL PVA solution), the theoretical value of the LLQ is 4 cells/m^3. To determine the LLQ, *A. fumigatus* IHEM 3007 spore suspensions with different concentrations were analyzed with the SPC procedure and plated. To that end, 1 mL of the suspension was added to 19 mL of pure PVA solution and 250 μL of the suspension was plated.

To check the linearity of the method, we sampled and analyzed different volumes (10−1000 L) of artificially contaminated air.

2.4. Evaluation of the Sensitivity and Specificity of the Assay. The sensitivity and specificity of the assay were determined by applying the SPC method to pure cultures. To that end, 1 mL of a spore suspension containing approximately 100 cells was filtered and microcolony formation and labeling were performed as described before (see section 2.1.). Spore suspensions were prepared for 40 *A. fumigatus* strains including IHEM 2494, IHEM 2952, IHEM 3007, IHEM 3768, IHEM 4184, IHEM 4185, IHEM 4187, IHEM 4189 (Instituut voor Hygiene en Epidemiologie, Brussels, Belgium), MUCL 978, MUCL 15821 (Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium), and 30 recent clinical isolates (CI) from our own collection. Additionally, cross reactivity was checked by applying the assay to 32 fungal strains, including several *Aspergillus* and *Penicillium* species that were chosen on the basis of their relatedness with *A. fumigatus* and/or their proven occurrence in air. A table with an overview of the different fungi used for specificity testing and their corresponding behavior is available in the Supporting Information.

2.5. Comparison between SPC and a Culture-Based Method for the Analysis of Air Samples. Air was artificially contaminated with *A. fumigatus* by opening a 3 day old culture plate to the air in a LAF cabinet (no agitation of the plate, LAF cabinet switched off). Subsequently, 100 L of air was collected using the MAS-100 *Eco* impaction air sampler onto three plates containing the PVA polymer and onto three plates containing 25 mL of SABc. For all 25 *A. fumigatus* strains used to contaminate the air (Table 1), the polymer and culture plates were sampled alternately, starting with a polymer plate. SABc plates were incubated at 47 °C; after 4 days, colonies were counted and a selection of colonies (maximum 21) was identified as *A. fumigatus* using light microscopy according to specific identification keys (*24*). No positive hole conversion was performed, as only a fraction of the colonies impacted and grown on the plate (only *A. fumigatus*) was enumerated. The SPC protocol was applied to samples collected onto the polymer plates.

In addition, triplicate 1000 L air samples from 23 various locations (Table 2) were collected and analyzed using the same approach.

Mann−Whitney and Kolmogorov−Smirnov tests were used to detect statistically significant differences ($p < 0.01$) between the results obtained with the culture-based method and SPC (SPSS 15.0.0, SPSS Inc.).

3. Results

3.1. Evaluation of the Capturing Efficiency of the PVA Film. No differences were observed between the colony counts after impaction on PVA and SABc for air samples contaminated with 10 different *A. fumigatus* strains, indicating that the impaction efficiency is the same for both media (Figure 1).

3.2. Determination of the Lower Limit of Quantification and Evaluation of the Linearity of the Assay. The quantification results obtained with plating and with the SPC

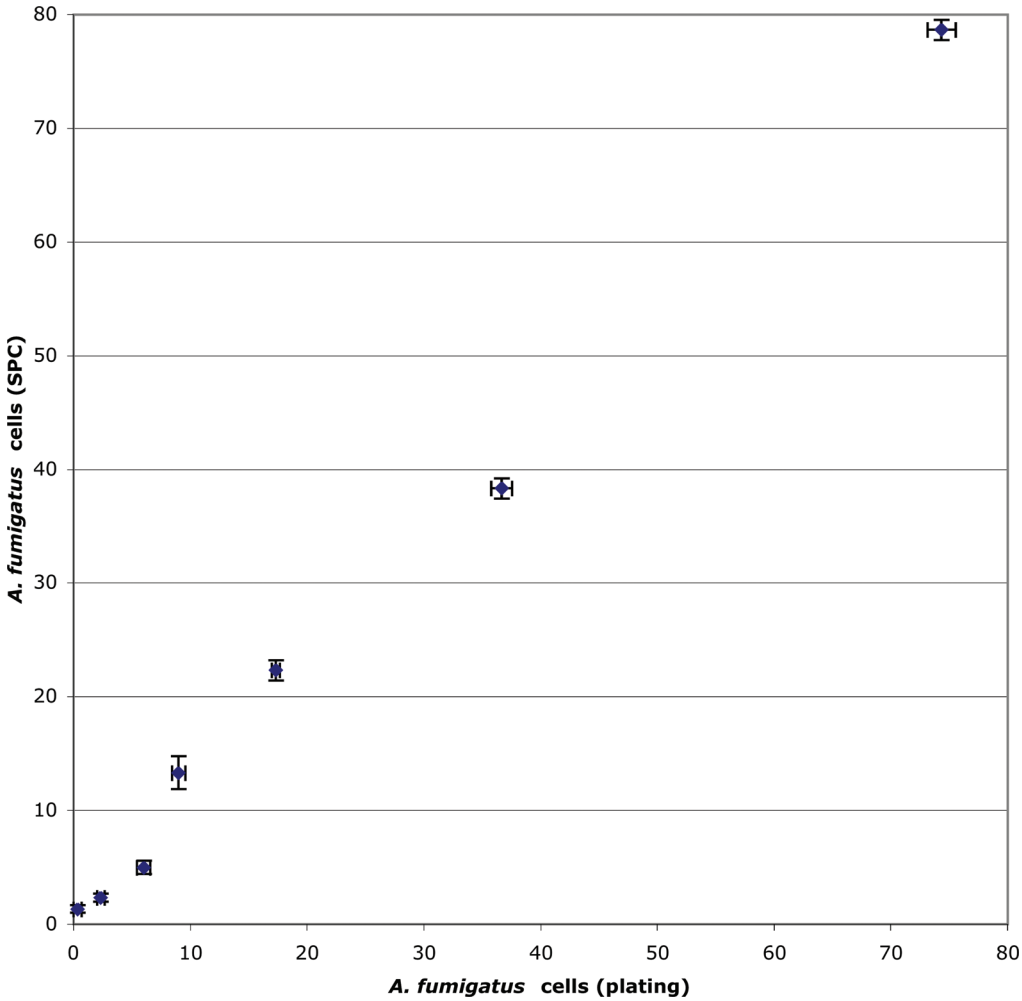


FIGURE 2. Correlation between the number of *A. fumigatus* cells obtained with the SPC procedure and plating for different cell concentrations.

procedure for different *A. fumigatus* concentrations are shown in Figure 2. The counts obtained with SPC ranged from 1.3 to 78.6 cells, whereas plate counts ranged 0.33 to 74.3 cells. No statistically significant differences between SPC and culture (Mann−Whitney test, $p < 0.01$) were found. As quantification of cell numbers as low as one cell per filter is perfectly possible with SPC, the theoretical LLQ (4 cells/m^3) is also the actual LLQ.

By analyzing different volumes of air samples, we were able to demonstrate the linearity of the method as a clear linear correlation between the volume of air sampled and the number of *A. fumigatus* cells can be observed (data available as Supporting Information). In contrast, previous results (*27*) demonstrated that the number of fungi/m^3 decreased as the sampling volume increased. However, this discrepancy may be explained by the resistance of *A. fumigatus* spores to desiccation during air sampling.

3.3. Evaluation of the Sensitivity and Specificity of the Assay. The appropriate level of sensitivity of the assay was confirmed, as all 40 *A. fumigatus* strains formed intensely labeled microcolonies that were detected by the Chemscan *RDI* and were retained after software discrimination (data not shown). Several of the fungi did not react with the antibody (*Absidia corymbifera*, *Alternaria alternata*, all *Fusarium* spp., *Mucor racemosus*, *Rhizopus microsporus*, and *R. oryzae*). However, multiple other fungi (all *Aspergillus* and *Penicillium* species tested, *Alternaria tenuissima*, *Paecilomyces variotii* and *Rhizopus stolonifer*) were also detected by the monoclonal anti-*Aspergillus* antibody. However, the growth step at 47 °C made sure that these fungi except *Paecilomyces variotii* and *Rhizopus stolonifer* were not codetected as no growth was observed at this temperature and labeled spores were excluded from the analysis based on software discriminant settings. Only *A. fumigatus*, *Paecilomyces variotii*, and *Rhizopus stolonifer* formed labeled microcolonies at this temperature. However, the morphology of these latter species is completely different from the characteristic morphology of *A. fumigatus* and the dichotomous branching of *A. fumigatus* hyphae makes rapid discrimination between these fungi during microscopic validation straightforward (Figure 3).

3.4. Comparison between SPC and a Culture-Based Method for the Analysis of Air Samples. The number of *A. fumigatus* cells in artificially contaminated air was determined by SPC and compared to results obtained with a culture-based method. In Table 1, the average number of *A. fumigatus* cells/m^3 is shown for the 25 strains used to contaminate the air. For SPC, *A. fumigatus* counts ranged from 200 to 20683 cells/m^3, whereas with the culture-based analysis, counts between 170 and 4000 (ULQ) cells/m^3 were obtained. For the artificially contaminated air samples, both methods yielded the same results ($p > 0.01$).

In Table 2, the average number of *A. fumigatus* cells/m^3 is shown for the 23 locations investigated. With SPC, *A. fumigatus* counts ranged from 5 to 57 cells/m^3, whereas with the culture-based analysis, counts between 0 and 18 cells/m^3 were obtained. For 14 locations, the number of *A. fumigatus* cells/m^3 found using the SPC procedure was below the LLQ for minimum one of the replicates. On the other hand, for five locations, culture plates were completely

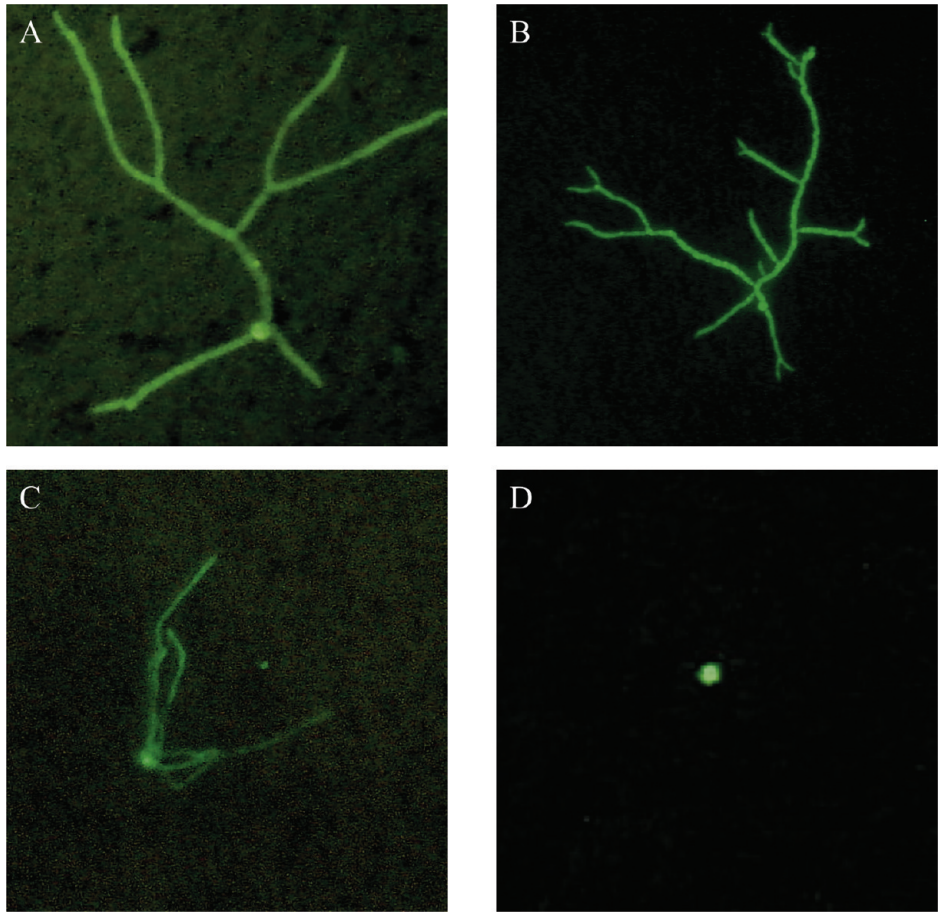


FIGURE 3. (A−C) Green fluorescent microcolonies and (D) spore after incubation at 47 °C for 18 h, antibody detection, and TSA. Microcolony of (A) *Aspergillus fumigatus* IHEM 3768, (B) *Aspergillus fumigatus* MUCL 978, and (C) *Rhizopus stolonifer* IHEM 4101. (D) Spore of *A. lentulus* IHEM 22458.

overgrown with a fungus different from *A. fumigatus*, making interpretation impossible. For all locations, the same results were obtained with both methods ($p > 0.01$).

4. Discussion

Results obtained with a new method based on SPC and immunofluorescence and a traditional culture-based method for artificially contaminated and environmental air samples were compared and proved similar in terms of microbial load. However, the SPC method has several advantages.

The LLQ of this SPC procedure is four *A. fumigatus* cells/m^3 if 1000 L of air is sampled. This LLQ is very low compared to other methods and meets the current requirements as according to recent guidelines (*35*), this LLQ (4 cells/m^3) equals the acceptance level for normal hospital rooms. The low number of *A. fumigatus* cells present in air neccessitates the collection of rather high volumes of air, leading to an increase in the number of other, potentially faster growing fungi in the sample. Although this does not affect the SPC analysis, the conventional culture plates may become overgrown as was demonstrated for five locations (Table 2). As this only becomes obvious after the incubation, resampling using a lower volume (and hence an increase of the LLQ) is the only possibility.

As the PVA solution can be diluted prior to analysis, the ULQ of the SPC procedure is adjustable. The ULQ for the culture-based method on the other hand is determined by the number of colonies on a single plate that can still be counted and microscopically examined. As the perforated lid of the air sampler consists of 400 holes, maximum 400 individual colonies can be obtained on the culture plate and the ULQ is therefore 4000 cells/m^3 for 100 L air samples. Although the low counts obtained for the air samples analyzed in this study do not indicate the need for a method with a high ULQ, several studies analyzing air samples taken during renovation or at specific sites (e.g., wood shipping facilities) report much higher concentrations (up to 1×10^6 *A. fumigatus* spores/m^3) (*14, 15, 21, 36*). Additionally, the much higher concentrations of *A. fumigatus* obtained in the artificially contaminated samples (Table 1) clearly show that large amounts of spores are released if air flows over a contaminated source.

A major disadvantage of the traditional culture-based technique is the long incubation period that is required. After tests with different media, we found that at least four days are necessary for *A. fumigatus* to form sporulating colonies with a distinct macroscopic and microscopic morphology (data not shown). In contrast, the SPC procedure takes only 24 h. Enhanced speed would improve infection control in hospitals and preventive measures (e.g., during construction work, at wood chipping facilities, etc.), as evidence-based corrective actions can be taken more rapidly (*17, 35*).

The skills necessary to identify *A. fumigatus* when the SPC protocol is used are minimal. Because of the combination of the microcolony formation at 47 °C and the immunofluorescent labeling, only *Paecilomyces variotii* and *Rhizopus stolonifer* need to be discriminated from *A. fumigatus* by microscopic validation. When a culture-based method is used, however, selective and differential media only allow a limited discrimination and subsequent microscopic analysis requires a profound knowledge of the morphology of a large variety of molds to obtain accurate identification.

New qPCR approaches (*25, 26*) used for the quantification of *A. fumigatus* are the most interesting, as they produce results very rapidly (less than four hours). However, these methods have a rather high LLQ of 90 cells per filter (necessary for DNA extraction), although this can be overcome by increasing the sampling time. However, a LLQ of 13 cells/m^3 was obtained only if the sampler was operated for eight hours (15 L/min), which drastically increases the time needed to obtain results wih this otherwise rapid method (*21, 25*). Additionally, air samples need to be collected using a specific, not widely used, and more expensive filter-based air sampler to allow subsequent DNA extraction from the filter.

To the best of our knowledge, this is the first report that combines SPC with immmunostaining in order to quantify cuturable *A. fumigatus* in air samples. Previously, some studies have used SPC and/or immunostaining for the detection of *A. fumigatus*. However, these studies either missed the quantitative aspect, as they used standard microscopy for detection (*37*), or depended on the validation step to detect the fluorescence obtained by immunostaining (*38, 39*). In the present study, the combination of immunodetection and the TSA method improved the fluorescence intensity, enabling direct detection of the labeled *A. fumigatus* microcolonies by the solid-phase cytometer. Additionally, the use of a PVA polymer allowed the use of a standard, cheap impaction sampler for the collection of airborne microorganisms.

Acknowledgments

We acknowledge the excellent technical assistance of Margherita Battista, Elise De Rocker, and Iris Clarysse. This research was financially supported by the BOF of the Ghent University (Project B/07601/02).

Supporting Information Available

Table with an overview of the different fungi used for specificity testing and their corresponding behavior; graph demonstrating the correlation between the volume of air sampled and the number of *A. fumigatus* cells obtained with the SPC procedure; figure with microscopic images of *A. fumigatus* and *R. stolonifer* microcolonies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Stetzenbach, L. D. Introduction to Aerobiology. In *Manual of Environmental Microbiology*, 3rd ed.; Hurst, C. J., Crawford, R. L., Garland, J. L., Lipson, D. A., Mills, A. L., Stetzenbach, L. D., Eds.; ASM Press: Washington, D.C., 2007; pp 925−938.

(2) Wu, F. Q. Culture-based analytical methods for investigation of indoor fungi. In *Sampling and Analysis of Indoor Microorganisms*; Yang, C. S., Heinsohn, P. A., Eds.; John Wiley & Sons: New York, 2007; pp 105−122.

(3) Gorny, R. L.; Reponen, T.; Willeke, K.; Schmechel, D.; Robine, E.; Boissier, M.; Grinshpun, S. A. Fungal fragments as indoor air biocontaminants. *Appl. Environ. Microbiol.* **2002**, *68*, 3522–3531.

(4) Curtis, L.; Cali, S.; Conroy, L.; Baker, K.; Ou, C.-H.; Hershow, R.; Norlock-Cruz, F.; Scheff, P. *Aspergillus* surveillance project at a large tertiary-care hospital. *J. Hosp. Infect.* **2005**, *59*, 188–196.

(5) Latgé, J.-P. *Aspergillus fumigatus* and aspergillosis. *Clin. Microbiol. Rev.* **1999**, *12*, 310–350.

(6) Rüchel, R.; Reichard, U. Pathogenesis and clinical presentation of aspergillosis. In *Aspergillus fumigatus: Biology, Clinical Aspects, And Molecular Approaches to Pathogenicity*; Brakhage, A. A., Jahn, B., Schmidt, A., Eds.; Karger: Basel, Switzerland, 1999; pp 21−43.

(7) Denning, D. W. Aspergillosis: diagnosis and treatment. *Int. J. Antimicrob. Agents* **1996**, *6*, 161–168.

(8) Denning, D. W. Invasive aspergillosis. *Clin. Infect. Dis.* **1998**, *26*, 781–805.

(9) Symoens, F.; Burnod, J.; Lebeau, B.; Viviani, M. A.; Piens, M. A.; Tortorano, A. M.; Nolard, N.; Chapuis, F.; Grillot, R. Hospital-acquired *Aspergillus fumigatus* infection: can molecular typing methods identify an environmental source. *J. Hosp. Infect.* **2002**, *52*, 60–67.

(10) VandenBergh, M. F. Q.; Verweij, P. E.; Voss, A. Epidemiology of nosocomial fungal infections: invasive aspergillosis and the environment. *Diagn. Microbiol. Infect. Dis.* **1999**, *34*, 221–227.

(11) Alberti, C.; Bouakline, A.; Ribaud, P.; Lacroix, C.; Rousselot, P.; Leblanc, T.; Derouin, F. Relationship between environmental fungal contamination and the incidence of invasive aspergillosis in haematology patients. *J. Hosp. Infect.* **2001**, *48*, 198–206.

(12) Falvey, D. G.; Streifel, A. J. Ten-year air sample analysis of *Aspergillus* prevalence in a university hospital. *J. Hosp. Infect.* **2007**, *67*, 35–41.

(13) Morris, G.; Kokki, M. H.; Anderson, K.; Richardson, M. D. Sampling of *Aspergillus* spores in air. *J. Hosp. Infect.* **2000**, *44*, 81–92.

(14) Cooper, E. E.; O'Reilly, M. A.; Dharmage, S. C. Influence of building construction work on *Aspergillus* infection in a hospital setting. *Infect. Control Hosp. Epidemiol.* **2003**, *24*, 472–476.

(15) Nihtinen, A.; Anttila, V.-J.; Richardson, M.; Meri, T.; Volin, L.; Ruutu, T. The utility of intensified environmental surveillance for pathogenic moulds in a stem cell transplantation ward during construction work to monitor the efficacy of HEPA filtration. *Bone Marrow Transplant.* **2007**, *40*, 457–460.

(16) Pini, G.; Faggi, E.; Donato, R.; Sacco, C.; Fanci, R. Invasive pulmonary aspergillosis in neutropenic patients and the influence of hospital renovation. *Mycoses* **2007**, *51*, 117–122.

(17) Bénet, T.; Nicolle, M.-C.; Thiebaut, A.; Piens, M.-A.; Nicolini, F.-E.; Thomas, X.; Picot, S.; Michallet, M.; Vanhems, P. Reduction of invasive aspergillosis incidence among immunocompromised patients after control of environmental exposure. *Clin. Infect. Dis.* **2007**, *45*, 682–686.

(18) Laumbach, R. J.; Kipen, H. M. Bioaerosols and sick building syndrome: particles, inflammation, and allergy. *Curr. Opin. Allergy Clin. Immunol.* **2005**, *5*, 135–139.

(19) Meyer, H. W.; Larsen, F. O.; Jacobi, H. H.; Poulsen, L. K.; Clementsen, P.; Gravesen, S.; Gyntelberg, F.; Norn, S. Sick building syndrome: association of symptoms with serum IgE specific to fungi. *Inflammation Res.* **1998**, *47*, S9–S10, suppl. 1.

(20) Ryan, T. J.; Whitehead, L. W.; Connor, T. H.; Burau, K. D. Survey of the Asp f 1 allergen in office environments. *Appl. Occup. Environ. Hyg.* **2001**, *16*, 679–684.

(21) Dillon, H. K.; Boling, D. K.; Miller, J. D. Comparison of detection methods for *Aspergillus fumigatus* in environmental air samples in an occupational environment. *J. Occup. Environ. Hyg.* **2007**, *4*, 509–513.

(22) Griffiths, W. D.; DeCosemo, G. A.L. The assessment of bioaerosols: a critical review. *J. Aerosol Sci.* **1994**, *25*, 1425–1458.

(23) Grinshpun, S. A.; Buttner, M. P.; Willeke, K. Sampling for airborne microorganisms. In *Manual of Environmental Microbiology*, 3rd ed.; Hurst, C. J., Crawford, R. L., Garland, J. L., Lipson, D. A., Mills, A. L., Stetzenbach, L. D., Eds.; ASM Press: Washington, D.C., 2007; pp 939−951.

(24) Klich, M. A. *Identification of common Aspergillus species*; Centraalbureau voor Schimmelcultures: Utrecht, The Netherlands, 2002.

(25) McDevitt, J. J.; Lees, P. S. J.; Merz, W. G.; Schwab, K. J. Development of a method to detect and quantify *Aspergillus fumigatus* conidia by quantitative PCR for environmental air samples. *Mycopathologia* **2004**, *158*, 325–335.

(26) Stetzenbach, L. D.; Buttner, M. P.; Cruz, P. Detection and enumeration of airborne biocontaminants. *Curr. Opin. Biotechnol.* **2004**, *15*, 170–174.

(27) Vanhee, L. M. E.; Nelis, H. J.; Coenye, T. Enumeration of airborne bacteria and fungi using solid phase cytometry. *J. Microbiol. Methods* **2008**, *72*, 12–19.

(28) Lisle, J. T.; Hamilton, M. A.; Willse, A. R.; McFeters, G. A. Comparison of fluorescence microscopy and solid-phase cytometry methods for counting bacteria in water. *Appl. Environ. Microbiol.* **2004**, *70*, 5343–5348.

(29) Mignon-Godefroy, K.; Guillet, J.-G.; Butor, C. Solid phase cytometry for detection of rare events. *Cytometry* **1997**, *27*, 336–344.

(30) Aurell, H.; Catala, P.; Farge, P.; Wallet, F.; Le Brun, M.; Helbig, J. H.; Jarraud, S.; Lebaron, P. Rapid detection and enumeration of *Legionella pneumophila* in hot water systems by solid-phase cytometry. *Appl. Environ. Microbiol.* **2004**, *70*, 1651–1657.

(31) Lemarchand, K.; Parthuisot, N.; Catala, P.; Lebaron, P. Comparative assessment of epifluorescence microscopy, flow cytometry and solid-phase cytometry used in the enumeration of specific bacteria in water. *Aquat. Microb. Ecol.* **2001**, *25*, 301–309.

(32) Engelhart, S.; Glasmacher, A.; Simon, A.; Exner, M. Air sampling of *Aspergillus fumigatus* and other thermotolerant fungi: comparative performance of the Sartorius MD8 airport and the Merck MAS-100 portable bioaerosol sampler. *Int. J. Hyg. Environ. Health* **2007**, *210*, 733–739.
(33) Yao, M.; Mainelis, G. Analysis of portable impactor performance for enumeration of viable bioaerosols. *J. Occup. Environ. Hyg.* **2007**, *4*, 514–524.
(34) Catala, P.; Parthuisot, N.; Bernard, J.; Baudart, J.; Lemarchand, K.; Lebaron, P. Effectiveness of CSE to counterstain particles and dead bacterial cells with permeabilised membranes: application to viability assessment in waters. *FEMS Microbiol. Lett.* **1999**, *178*, 219–226.
(35) Fenelon, L. *National Guidelines for the Prevention of Nosocomial Invasive Aspergillosis during Construction/Renovation Activities*; National Disease Surveillance Centre: Dublin, Ireland, 2002.
(36) Streifel, A. J. Aspergillosis and construction. In *Architectural Design and Indoor Microbial Pollution*; Kundsin, R. B., Ed.; Oxford University Press: New York, 1988; pp 198−217.
(37) Green, B. J.; Schmechel, D.; Sercombe, J. K.; Tovey, E. R. Enumeration and detection of aerosolized *Aspergillus fumigatus* and *Penicillium chrysogenum* conidia and hyphae using a novel double immunostaining technique. *J. Immunol. Methods* **2005**, *307*, 127–134.
(38) De Vos, M. M.; Nelis, H. J. Detection of *Aspergillus fumigatus* hyphae by solid phase cytometry. *J. Microbiol. Methods* **2003**, *55*, 557–564.
(39) De Vos, M. M.; Sanders, N. N.; Nelis, H. J. Detection of *Aspergillus fumigatus* hyphae in respiratory secretions by membrane filtration, fluorescent labelling and laser scanning. *J. Microbiol. Methods* **2006**, *64*, 420–423.

ES803435A

Manuscript ID: es-2008-03435a

Title: "**Rapid detection and quantification of *Aspergillus fumigatus* in environmental air samples using solid-phase cytometry**"

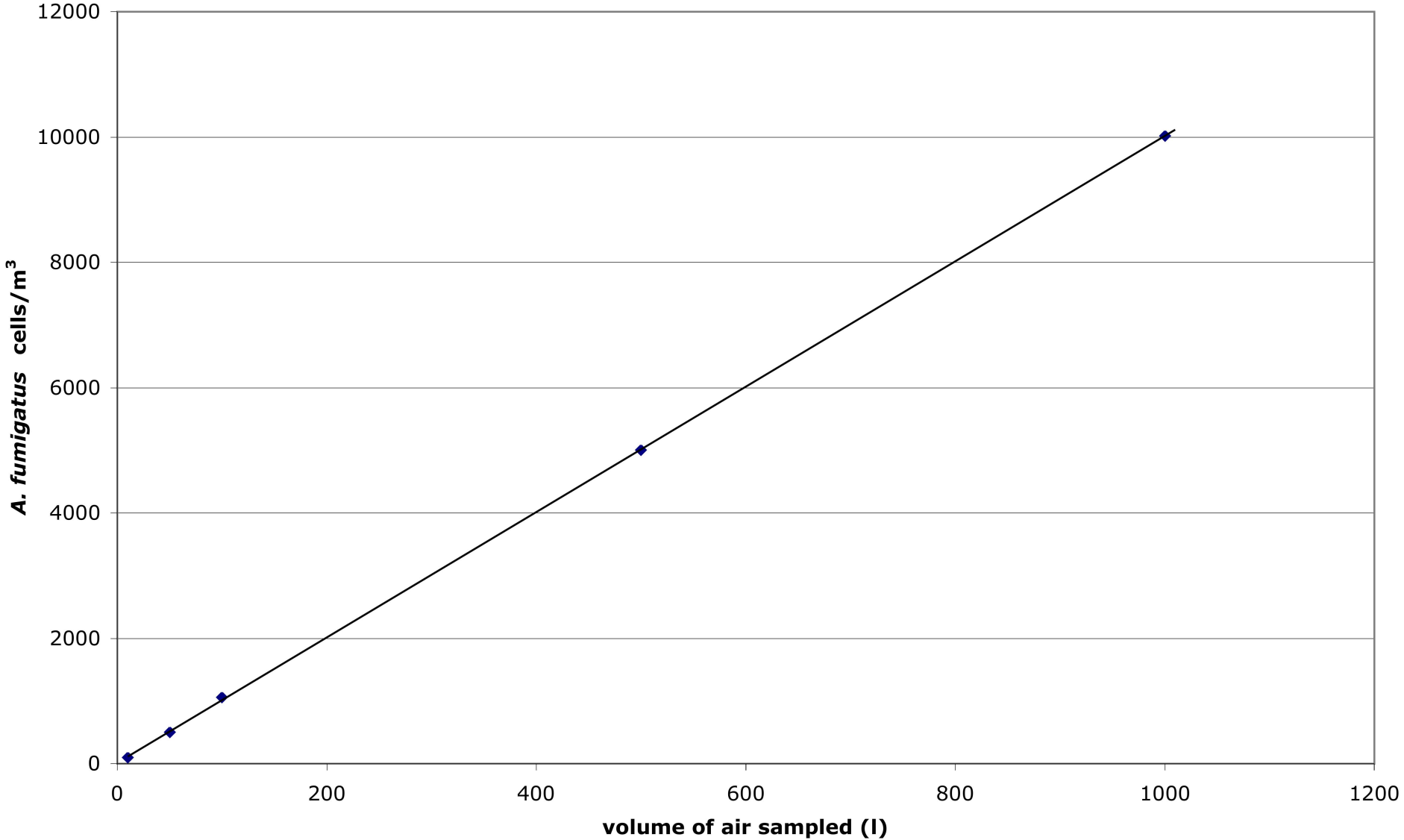
Author(s): Vanhee, Lies; Coenye, Tom; Nelis, Hans

Table: Overview of the fungi used for specificity testing with their corresponding behaviour in the assay.

Figure S1: A graph demonstrating the correlation between the volume of air sampled and the number of *A. fumigatus* cells obtained with the SPC procedure is also available.

Table: Overview of the fungi used for specificity testing with their corresponding behaviour in the assay.

Fungus	Strain number (s) [a]	Microcolony formation (47 °C, 18 h)	Cross reactivity with the anti-*Aspergillus* AB
Aspergillus clavatus	IHEM 5138, IHEM 9776	-	+
Aspergillus flavus	IHEM 2700, IHEM 6741	-	+
Aspergillus lentulus	IHEM 22458, IHEM 22459	-	+
Aspergillus nidulans	IHEM 3563, IHEM 4190	-	+
Aspergillus niger	IHEM 4781, IHEM 9673	-	+
Aspergillus terreus	IHEM 349, IHEM 3280	-	+
Aspergillus versicolor	IHEM 2157, IHEM 2646	-	+
Penicillium aurantiogriseum	IHEM 6620	-	+
Penicillium brevicompactum	IHEM 1388	-	+
Penicillium capsulatum	IHEM 1376	-	+
Penicillium chrysogenum	IHEM 3035	-	+
Penicillium citrinum	IHEM 1328	-	+
Penicillium commune	IHEM 10238	-	+
Penicillium crustosum	IHEM 6359	-	+
Penicillium digitatum	IHEM 3201	-	+
Penicillium duclauxii	IHEM 3498	-	+
Penicillium italicum	IHEM 3200	-	+
Penicillium piceum	IHEM 3036	-	+
Penicillium roqueforti	IHEM 3196	-	+
Penicillium simplicissimum	IHEM 1202	-	+
Penicillium spinolosum	IHEM 970	-	+
Absidia corymbifera	IHEM 5092	+	-
Alternaria alternata	IHEM 2505	-	-
Alternaria tenuissima	IHEM 3327, IHEM 4179	-	+
Fusarium graminearum	IHEM 2994	-	-
Fusarium oxysporum	IHEM 14513	-	-
Fusarium solani	IHEM 6743	-	-
Mucor racemosus	IHEM 325	-	-
Paecilomyces variotii	IHEM 3285, IHEM 14443	+	+
Rhizopus microsporus	IHEM 7978	+	-
Rhizopus oryzae	IHEM 6016	+	-
Rhizopus stolonifer	IHEM 718, IHEM 4101	+	+


12000
10000
8000
6000
4000
2000
0
A. fumigatus cells/m3
0
200
400
600
800
1000
1200
volume of air sampled (l)