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Performance of UVAPS with respect to detection of airborne fungi

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Abstract

This paper studies the detection limit, selectivity and counting efficiency of an Ultraviolet Aerodynamic Particle Sizer Spectrometer (UVAPS) with regard to aerosolized fungal spores. The study demonstrated the ability of the instrument for detection and measurement of fungal spores under controlled conditions. A reasonable correlation was found between the UVAPS and the AGI-30 impinger in measuring the aerosol fungal spore concentrations under investigation: *Penicillium* and *Aspergillus niger* (r = 0.911, p < 0.005 and r = 0.882, p < 0.05, respectively). A linear relationship between total particle concentration and fluorescent particle concentration was found in the range from 0 to 70 particles/cm³. Its lower detection limit was found to be 0.01 particles/cm³. The dry generation method which was used for generating fungal spores has proved to be reproducible and easy to control, as well as simple and inexpensive

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1. Introduction

There is increasing scientific and medical evidence that exposure to biological aerosols has significant health implications (Dales et al., 1991; Koskinen et al., 1997; Verhoeff and Burge, 1997). Diseases like tuberculosis, diphtheria and legionellosis are caused by infectious microorganisms that are transmitted via airborne routes and deposited in the respiratory tract (Maus et al., 2001). Microbial spores and other microbial components can cause hypersensitivity in susceptible people (Nester et al., 2004). Fungi are responsible for many allergic conditions in humans such as asthma, rhinitis, allergic bronchopulmonary mycoses and hypersensitivity pneumonitis (Vijay et al., 1999). Infection is another way for fungi to affect human health. Dermatomycosis and aspergillosis are two infectious diseases that are caused by molds and *A. fumigatus*, respectively (Smith, 1976). Some mold infections, although rarely impacting healthy individuals, may be fatal for those suffering immunodeficiency, or recovering from burns or surgery (Kowalski, 2000).

Viable and non-viable fungal particles are associated with health effects (Samson et al., 1994). Viability is a prerequisite for bioaerosols to be infectious; although it is not a prerequisite in causing allergenic or toxic effects (Willeke and Baron, 1993). It has also been reported that fungal spores release allergens during their germination (Mitakakis et al., 2001; Green et al., 2003). For the above reasons, viable spores are emphasized in this investigation as more significant than total spore measurements. However, the effects cause by toxins or beta-glucan are not dependent on spore viability (Bhatnagar et al., 2005).

Real-time detection and identification of bioaerosols has become desirable in medical and agricultural areas, as well as in situations where risk is high. Real-time monitoring has many advantages over conventional methods, including brief sample detection (in the order of seconds), reduced labour and greater analytical frequency due to the possibility of continuous monitoring.

The Ultraviolet Aerodynamic Particle Sizer (UVAPS, model 3312, TSI, St. Paul., MN) can provide concentrations, size distributions, and fluorescence for particles with aerodynamic diameters of 0.5 to 15 μ m, in real-time. Fluorescence detection is performed by exciting particles with an UV laser beam at 355 nm and then detecting emissions at 420 to 575 nm.

Viable cells of most organisms, including fungi, have a natural auto-fluorescence due to biochemical fluorophores such as the reduced fluorescent coenzymes nicotinamide-adenine dinucleotide (NADH) and nicotinamide-adenine dinucleotide phosphate (NADPH), the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), and metabolic function riboflavin (vitamin B₂) (Li et al., 1991; Brosseau et al., 2000; Billinton and Knight, 2001). The maximum excitation and emission peaks for the coenzymes NAD(P)H are 360 nm and 460 nm, respectively (Dellinger et al., 1998; Billinton and Knight, 2001). Flavins' maximum excitation peaks are 360 nm and 445 to 470 nm (Dellinger et al., 1998) or 385 nm (Li et al., 1991), with a maximum fluorescence peak at 530 nm (Billinton and Knight, 2001) or 525 nm as in Li et al. (1991). NAD(P)H and flavins are largely responsible for fungal fluorescence detected by the UVAPS.

While many studies have been conducted to detect bacteria using the UVAPS (Brosseau et al., 2000; Agranovski et al., 2003a; Agranovski et al., 2003b), very limited research has been done on evaluating the method for monitoring airborne fungi. The primary goals of this work were to determine the abilities and limitations of the UVAPS with respect to detection of fungal aerosols. The study was also

4

focused on investigating the possibility of discriminating between different fungi using the UVAPS technology. It also introduces a simple and reproducible method for generating airborne fungal spores.

2. Materials and methods

All experiments were conducted in a Class II, Type A, Biological Safety Cabinet (SG-400 SterilGARD, E-mail Westinghouse Pty Ltd., Australia) in the International Laboratory for Air Quality and Health (ILAQH).

2.1. The UVAPS calibration

The UVAPS is designed to record particles with respect to their fluorescence in channels 1 to 64. The particles with no fluorescent compounds appear in channel 1 and in a very small fraction in channel 2; while the bioaerosols (with endogenous metabolites) appear in the channels from 2 to 64, according to their fluorescent intensity. The particles with higher fluorescent intensity are recorded in the higher channels (TSI-Incorporated, 2000).

The fluorescence spectra detected by the UVAPS are very sensitive to change in the UV laser pulse energy and photomultiplier tube (PMT) gain, i.e. doubling the quantity of each of the two parameters would lead to doubling of the measured fluorescence intensity (TSI-Incorporated, 2000; Agranovski et al., 2003a). In this work, the detected threshold base line was controlled and checked during the course of the experiments. The UV laser pulse energy and the PMT gain were set to $50 \pm 1\%$ of the laser's full power and 482 V, respectively. At such settings, 0.993 µm diameter monodispersed Polystyrene Latex (PSL) Particles (Duke Scientific Corporation, Palo Alto, CA) started to give weak fluorescent signals in channel 2, while 0.91 μ m diameter Blue Fluorescent (BF) microspheres (Duke Scientific Corporation, Palo Alto, CA) appeared in the last channel.

The UVAPS was calibrated using 0.993 μ m diameter monodispersed Polystyrene Latex (PSL) particles and 0.91 μ m diameter Blue Fluorescent (BF) microspheres, which were aerosolized with a Collison nebuliser. Suspensions were made by diluting one drop from stocks in sterilized, distilled water to concentrations in the order of 10⁷ particles/mL. Monosized polymer particle suspension, 0.1% solids (SS-2-PXG 0.1%), (SS-5-PXG 0.1%) and (SS-7-PXG 0.1%) standards with 1.05 g/cm³ from DYNO PARTICLES AS were also used for calibration of the UVAPS. The factory calibration procedure (dry redispersion) was used for these standard particles.

2.2. Sample preparation

2.2.1. Fungal species used for aerosolization by Collison nebulizer

Penicillium sp. (Australian Collection of Microorganisms - ACM 4616) was inoculated on Sabouraud Dextrose Agar (SDA) and incubated at 25°C for one week. A suspension of spore solution was prepared by scraping the surface of the *Penicillium* mycelia with a sterilized needle (without touching the agar) and transferring the spores to the Collison nebulizer jar containing deionized water. *Aspergillus niger* (American Type Culture Collection - ATCC 9142) prepared in the same way as *Penicillium* sp. The measurements of background concentrations of aerosols generated from distilled water were conducted before each experiment. In addition, to control the effect of contamination of the samples with SDA during surface scraping of the mycelia, the blanks of SDA (a few grams in distilled water) were also tested three times. The measurements of SDA blanks were conducted in order to evaluate the fluorescent signal of agar, if any, that could contribute to the total signals when the suspensions of fungal spores were aerosolized. Collison

2.2.2. Fungal species used for aerosolization by dry (direct) method

Penicillium sp. (ACM 4616) and *Aspergillus niger* (ATCC 9142) were inoculated on SDA and incubated at 25°C for one week, then refrigerated for one day before being used in a direct aerosol generation method. The fluorescent percentage of fungal spores was found to decrease as they aged, as well as with an increased frequency of air exposure (Kanaani et al., 2007), and as such, these samples had a higher fluorescent percentage because they were young colonies and had not been exposed to air currents. They are subsequently referred to as having a "high fluorescent percentage". In addition, cultures with a "low fluorescent percentage" were used for comparison of the UVAPS performance with the AGI-30 impinger (AGI-30 as described in section 3.3.3.), which was used as a reference biosampler. These cultures were obtained as follows. After using the "high fluorescent percentage" cultures of *Penicillium* sp. and *A. niger* in the experiments, the agar plates with fungal cultures were refrigerated for three months and then used again for generating fungal aerosols with a "low fluorescent percentage".

2.3. Microscopic analysis

A light microscope (Model CX31RTSF, Olympus Corporation, Tokyo, Japan) was used to investigate the fungal particles. Pieces of transparent adhesive tape were distributed on the floor of the mixing chamber; while others were placed on the chamber wall, close to the inlet sampling ports, so that the sticky side was facing towards the released particles (Figure 2). In addition, uncoated microscope slides were distributed on the chamber floor. After fungal particles were generated, each tape piece was mounted onto a microscope slide with the sticky side down and viewed together with uncoated slides under a microscope, using the 40x objective (400x magnification). These tests were performed three times for both *Penicillium* sp. and *A. niger* samples generated by the direct method.

2.4. Experimental set-up and procedures

2.4.1. Generation of fungal aerosols with a Collison nebulizer

As shown in Figure 1, aerosols were generated using a 6-jet Collison nebulizer (BGI Inc., Waltham, MA), which was operated at a flow rate of 7 L/min. Supply compressed air was filtered with a HEPA filter. Droplets carrying microorganisms were dried by a silica gel dryer, before entering the mixing chamber with compressed HEPA-filtered air, supplied at a flow rate of 4 L/min. A mixing chamber was used to provide the same physical sampling points for all tests to achieve homogenous particle distribution and to control the concentration of aerosols inside the chamber. The dimensions of the mixing chamber were $100 \times 39 \times 39$ cm and it was made of aluminium except for one side, made of Perspex, used as a door.

2.4.2. Dry (direct) spore generation method

In the dry generation method, the fungi were aerosolized directly from the cultures growing on the agar plates, which were placed inside of the chamber (Figure 2). During experiments, compressed HEPA-filtered air was entered the mixing chamber at a flow rate of 10 L/min. The continuous air flow contacted the surface of the *Penicillium* or *A. niger* mycelia at an angle of 60° and a distance of 1.5 cm.

Measurements of the total and the fluorescent particle counts were conducted using the UVAPS.

Simultaneously, fungal aerosols were sampled with the AGI-30 impingers. The exhaust airflow from both the UVAPS and the AGI-30 were HEPA-filtered and then returned to the biosafety cabinet, for safety and to prevent contamination of the experimental area (Figures 1 & 2). In all the experiments the UVAPS sample time was 20 s.

2.4.3. Operational procedures

The UVAPS operated at 5 L/min and the AGI-30 impinger at 12.5 L/min, while the inlet air was supplied at 10 L/min. Hence a shortfall of inlet air came from two holes used as "inlet-outlet equalizing holes", located on the sides of the chamber (Figure 2). As the chamber was placed inside of the biosafety cabinet, the make-up HEPA-filtered air was taken directly from the cabinet. The concentrations inside the chamber were measured prior to each experiment and it was confirmed that the background concentrations were mostly 0.0 #/cm³. Short inflexible tubing was used on sampling ports to minimise particle losses. The trials in these experiments took place at 23-25°C and 51-60% relative humidity. This is a moderate humidity range, in which the spores remain as singlets and do not aggregate (Reponen et al., 1996). Room temperature was used in order to represent a typical indoor environment and also because temperature plays an insignificant role in the hygroscopic growth of the particles (Li and Hopke, 1993). To obtain statistically significant results, the experiments were conducted seven times for each organism.

The AGI-30 impingers with the collection medium were autoclaved before being used in the tests. The aerosolized fungal spores passed through a jet into a collection medium of 20 ml of 0.1% peptone water with 0.01% Tween 80 and 0.005% antifoam

9

Y-30 emulsion (SIGMA-ALDRICH, Inc., St. Louis, Mo.). The 0.01% Tween 80 and 0.005% antifoam were added to improve the empinger efficiency as shown in section 3.3.3. The sampling times were 10 and 20 minutes for high and low concentration, respectively. After finishing each experiment, the neck of impinger was flushed with the impinger solution. The final volume of each impinger was measured and corrected for evaporation. The AGI samples were brought to a final volume of 100 ml with sterile 0.1% peptone water. Then 0.1, 0.2 and 0.4 ml of each sample were plated onto duplicate SDA plates and then incubated at 25°C. *Aspergillus* colonies were counted after 48 hours and *Penicillium*, which grows slower, after 60 hours. The concentrations in CFU/m³ were calculated from the colony numbers, the dilution factor and the volume of sampled air. Blanks were collected and treated in the same way as fungal samples at each trial, except that the petri dish was contained SDA only instead of SDA with the interested fungal colonies. Background concentrations were also measured at each trial with the UVAPS.

3. Results and Discussion

3.1. The UVAPS calibration

Monodispersed Polystyrene Latex (PSL) $0.993 \,\mu\text{m}$ diameter particles and $0.91 \,\mu\text{m}$ diameter Blue Fluorescent (BF) microspheres were used at $1.05 \,\text{g/cm}^3$, and their aerodynamic diameters were 1.02 and 0.93, respectively (Willeke and Baron, 1993). After these standards were aerosolised with the Collison nebulizer, the mean aerodynamic diameters were 1.0 μm and 0.90 μm , as measured by the UVAPS (Figs 3b, 3c and Table 2).

As mentioned above, standard particles of SS-2-PXG 0.1%, SS-5-PXG 0.1% and SS-7-PXG 0.1% with 1.05 g/cm³ (corresponding to aerodynamic diameters of 2.05, 5.12 and 7.17 μ m, respectively) were also used for calibration. Mean aerodynamic diameters of 1.99, 4.98 and 6.98 μ m were obtained, which all fell within the diameter accuracy provided by the supplier (± 3%). The range of the standard particles which were used for calibration covered the entire size range of fungal aerosols under investigation.

In regard to the fluorescence signals, the UVAPS showed selectivity for the special materials used in this study. While BF microspheres exhibited fluorescence of 97.6%, the PSL showed 0.13% (Table 2). The normal blank and SDA blank showed a fluorescence of 0%. The concentrations of BF and SDA aerosols were 22.1 and 18.9 $\#/cm^3$, respectively. The concentration of distilled water blank aerosol generated by Collison nebulizer was very low (0.13 $\#/cm^3$ with mean diameter of 2µm for a 20 sec sampling time). This concentration was negligible in comparison with the concentration of the standards.

3.2. Application of Collison nebulizer for generating fungal aerosols

Collison nebulizer was not an effective means for generating fungal aerosols for two reasons. Firstly, it was unable to generate particles large enough to cover all the fungal particles under investigation, which can reach up to 6.7 μ m in Aspergillus niger (see Figure 6b), and hence, a proper comparison between UVAPS and AGI-30 would not be able to be established. This was confirmed by testing a series of standard particles. While standard particles of 2 μ m in diameter (SS-2-PXG 0.1%) were atomized by the nebuliser and detected easily (Figure 4a), standard particles of 5 μ m in diameter (SS-5-PXG 0.1%) gave no signal using the same method. A mixture of (SS-2-PXG 0.1%), (SS-5-PXG 0.1%), (SS-7-PXG 0.1%), (SS-10-PXG 0.1%) and (SS-15-PXG 0.1%) was also measured using same method and only a signal for $2 \,\mu m$ was detected (Figure 4b). Secondly, there was a non-homogeneous distribution of fungal particles in the solutions, due to the hydrophobic nature of the fungi. These two factors contributed to the failure of the instrument in detecting the Aspergillus niger and Penicillium sp in the proper way. Although signals were obtained for Penicillium sp (Figure 4c), they were not reproducible due to the non-continuous spore generation. The Collison nebulizer (BGI Inc., Waltham, MA) produces aerosols with a mass median diameter of 2 µm and a geometric standard deviation of 2 (Willeke and Baron, 1993). However there are many recent studies which produce fungal spores with mean physical dimensions of 1.8, 2.5, and 3 µm (Grinshpun et al., 2007) or fungal spores with mean aerodynamic diameters up to 3.1 µm (Yao and Mainelis, 2006) using a 6-jet Collison nebulizer (BGI Inc., Waltham, MA). More investigations are needed to determine the cut-off size of this nebuliser and its efficiency with regard to each aerosolised particle size. Since no positive results were obtained for generating fungal particles by this method, the tests were further conducted using only the dry (direct) generation method.

3.3. Direct generation method

3.3.1. Fungal particles morphology

To identify whether the released fungal particles are spores, hyphal fragments or fungal fragment propagules, the particles were investigated by a light microscope.

Based on the particle characteristics such as shape and size, the particles were classified as spores (Table 1); mostly singlets with a small percentage of spore chains

consisting of two or more spores. Fungal hyphae were not clearly observed on the slides. On the other hand, similar size distribution and almost the same mean aerodynamic diameters (Table 1) were detected by the UVAPS for all of the samples tested during the course of these experiments of samples, which indicated the release of fungal spores. The mean aerodynamic diameters measured with the UVAPS were in an agreement with literature data for the size of spores under investigation. It is typical to find most of the airborne fungi of these sizes in spore form, because spores are dry and easily released by airflow, especially those at the end of the conidial chains (Ingold, 1979). While hyphal or spore fragments were not detected at the lower flow rates, at the higher flow rates such as 15, 20, 25 and 30 L/min (corresponding to 5.5, 7.1, 8.5 and 10.2 m/s) fragmentation did occur; with a fragmentation percentage of 0.98%, 2.7%, 7.9% and 8.1%, respectively. The fragmentation percentage is defined as the number of samples which contained fragmented spores as a percentage of the total number of samples at each flow rate.

3.3.2 Fungal spore aerosolization

The direct particle generation method showed a distinct difference between the blank and fungal samples (Figures 3a, 5a & 5b). It has been shown that when the Collison nebulizer is used for generating bacterial aerosols from non-microbial blanks (e.g., agar washing, peptone water, broth media), it produces relatively strong fluorescent signals (Agranovski *et al.*, 2003a). In such cases, the UVAPS is not selective for the specific microbial fluorescent molecules (Agranovski *et al.*, 2003a). In the dry generation method of producing fungal aerosols this defect can be avoided, as fungal spores are generated directly from the agar plates with very little or no effect from the growth medium (measured as a background). Thus, under controlled conditions, the UVAPS was selective towards the fungal spores under investigation. The fluorescence signals were quite different for the fungal and the blank aerosols, as well as for the fungal genera examined (*Aspergillus* and *Penicillium*), through the fluorescent percentage and, to a lesser extent, their sizes (see section 3.3.4.). The concentrations for the blanks (aerosols generated from sterile agar plates without fungi) were up to 0.1 #/cm³ and the fluorescent percentage was zero.

The UVAPS determined the abundance and size characteristics of spores for each fungus tested. As shown in Table 1, the fungal spore size characteristics obtained with the UVAPS were in agreement with the literature data.

For *Penicillium* sp., 98% of the total fluorescent particles showed fluorescence signals in the range of 2.1-4.0 μ m with the remaining 2% distributed between 1.8-2.0 μ m and 4.0-4.7 μ m (Figure 6a). For *A. niger*, 70% of fluorescent particles had aerodynamic diameters in the range of 2.5-4.1 μ m, more than 99% were between 2.3-5.8 μ m, while 1% were detected in the ranges of 2.0-2.2 μ m and 5.9-6.7 μ m (Figure 6b).

These low percentages of large particles (around 6.7 μ m for *Aspergillus* and 4.7 μ m for *Penicillium*) are likely a result of aggregation due to the collision of smaller particles, which increased at high concentrations, and due the fact that the minority of particles were chains of two or more spores.

3.3.3. UVAPS and AGI-30 correlation

It has been suggested that both the microbial Andersen impactor and the AGI-30 impinger are suitable reference samplers for measuring culturable bioaerosols (Willeke and Macher, 1999). The Andersen impactor was not used in this study because the total concentrations of fungal particles were very high (in the order of 10^{6} - 10^{7} particles/m³), which would cause rapid (in less than one minute) overloading of the agar plates (Thorne et al., 1992). On the other hand, the AGI-30 was reported to

be suitable for fungal assays (Cox and Wathes, 1995). Thorne et al. (1992) found that the AGI-30 is the preferred sampling method for viable fungi in swine barns where it was reported that Penicillium, Cladosporium and Aspergillus (which are hydrophobic in nature) are the dominant fungal species (Chang et al., 2001). In addition, Awad (2002) used the AGI-30 impingers for sampling viable fungi in the environments where Penicillium, Cladosporium and Aspergillus species were the dominant fungi. However, Cage et al. (1996) reported that hydrophobic fungal spores may be collected inefficiently, but the addition of surfactant would improve the capture and retention of these hydrophobic spores. Therefore to improve the impinger efficiency, the following measures were applied in this study: (1) sampling times were 10-20 minutes, to minimize the vaporization (the decreasing volume was 1.1 ± 0.2 ml and 1.9 ± 0.2 ml, respectively) and to decrease particle bounce (the 10-20 minute sampling time is within the optimal time range for the instrument, so the impinger bioefficiency for collecting fungal spore is high (Lin and Li, 1998) and although a longer sample interval would be more representative fungal assay, the physical collection efficiency for the size range of particles under investigation is significantly reduced after one hour sampling, due to the evaporation of liquid and to particle reaerosolization (Grinshpun et al., 1997)); (2) Tween 80 (surfactant) was added to the collection liquid to improve the capture and retention of these hydrophobic spores; (3) antifoam was added to decrease bubbles and consequently to decrease particles reaerosolization; and (4) 20 mL of the collection liquid was used to enhance the sampling efficiency of the impinger (Cage et al., 1996; Grinshpun et al., 1996).

As shown in Figures 7a & b, the UVAPS and the AGI-30 samplers showed a reasonable correlation for both *Penicillium* and *A. niger* (r = 0.911, p < 0.005 and r = 0.882, p < 0.05, respectively). The UVAPS showed a low standard deviation of

fluorescent percentage for both high and low fluorescence cultures (81.5 ± 1.6 and $25.1 \pm 0.5\%$ for *Penicillium* and 62.4 ± 1.1 and $15.3 \pm 0.8\%$ for *A. niger*) compared to the culturable percentage obtained by the AGI-30 (67.25 \pm 15.5 and 22.0 \pm 2.5% for *Penicillium* and 50.5 \pm 14.0 and 16.8 \pm 1.8% for *A. niger*). The concentrations obtained with the AGI-30 were smaller than those by the UVAPS, which may be due to the hydrophobic nature, culturability and viability of the spores under investigation (since the UVAPS is measuring viable spores whereas the results from AGI-30 are culturable spores). There was one exception when the results of the AGI-30 (for A. niger) were higher than for the UVAPS (16.8 and 15.3%, respectively). This may be explained by the separation of cluster spores from single spores in the collection fluid of the AGI-30 (Willeke and Macher, 1999); however, this issue needs more investigation. The concentrations of total particles generated during the experiments were 8-16 #/cm³ for *Penicillium* and 4-13 #/cm³ for *A. niger* for a high fluorescent percentage samples, and around 2 and 3 #/cm³ for *Penicillium* and *A. niger* for low fluorescent percentage samples, respectively. The culturable percentage of blanks was zero for both *Penicillium* and *A. niger* in all trials.

Such correlation may be due to the UVAPS and the AGI-30 impinger both collecting aerosols from the same zone (sampling ports were in close proximity). Figure 8 also shows a linear relationship between the total and fluorescent particle concentrations. The high linearity shows the homogenous distribution of fluorescent biomolecules over the population of fungal spores. Table 2 also shows that the UVAPS behaved in a selective manner towards the fungal spores. The fluorescence signals of both species may be due their bimolecular (NADH, NADPH and riboflavin) content.

3.3.4. The potential of the UVAPS in differentiating between fungal species

All fungal spores contain the same sources of autofluorescence such as NADH, NADPH and riboflavin (Li et al., 1991; Brosseau et al., 2000; Billinton and Knight, 2001). However, the fluorescence signals of these biomolecules may vary due to their concentrations and the environmental conditions under which the fungal colonies are placed.

For high fluorescence cultures, the average percentage fluorescence for Penicillium was $81.5 \pm 1.6\%$ and for A. niger the average was $62.4 \pm 1.1\%$; while for low fluorescence cultures, the percentage was 25.1 \pm 0.5 % for *Penicillium* and 15.3 \pm 0.8% for A. niger (Figure 9 and Table 2). This suggests that the spores of Penicillium species have more riboflavin and/or NAD(P)H than those of A. niger, as both were exposed to the same culturing and test conditions. It was also found that the fluorescent percentage of spores increases with increasing aerodynamic diameter within the species (Kanaani et al., 2007), which indicates that larger particles are metabolically more active. On the other hand, *Penicillium* aerosols, which had a mean spore size smaller than that for Aspergillus aerosols, had larger fluorescent percentage than Aspergillus aerosols. This may suggest a difference in the fluorescent properties (e.g. amount of fluorophores) of viable spores for these two species or that there is a difference in the fraction of viable spores between these two species. It may also be due to other reasons which need further investigation. In regard to the spore size, although the mean aerodynamic diameters of these fungi were close to each other, they were not identical: $2.53 \pm 0.10 \ \mu m$ for *Penicillium* sp. and $3.51 \pm 0.08 \ \mu m$ for *A*. niger (Tables 1 and 2). These two aerosols also differed in their size distribution range (Figure 6a&b). In the entire study (which investigated thousands of samples), not one spectra for the *Penicillium* samples was similar to the *Aspergillus* samples.

The UVAPS has demonstrated the ability to sort fungi (*Penicillium* and *A. niger*) by fluorescence percentage and size when cultures were exposed to the same growth conditions. On the other hand, it has to be noted that the UVAPS would not sort these two fungi in the field (ambient air samples). It has been shown that the percentage fluorescence of *A. niger* and *Penicillium* sp. decreases with the spore age and frequency of air exposure (Kanaani et al., 2007). It has been also reported Huber et al.,(2000) that the fluorescence spectra for free NADH and those bound to protein are different. So in the field, where microorganisms are subjected to different environmental conditions (such as air speed) and due to the presence of a mixture of microorganisms of varying ages, the fluorescence signals would not be useful in differentiating between fungal spores. In addition, the presence of non-biological aerosols, which may also fluoresce at the same wave length (355nm) as spores, will add to the difficulties of differentiating microorganisms in environmental samples.

3.3.5. The relationship between total and fluorescent particle concentrations

The total and the fluorescent particle concentrations measured by the UVAPS showed a linear relationship until the concentrations of total particles reached 70 particles/cm³ for both species (Figure 10). Beyond this point, the graph ceases to be linear and total particle number increases at a greater rate than fluorescent particle number. This can be explained by considering the UVAPS as two distinct parts, the APS part (for counting the total particles) and the UV laser part (for counting the fluorescent particle). When a particle passes through two constant red laser beams, the scattered light is collected on the photodiode detector and the APS part counts one particle. The time needed for the APS to count another particle is less than that needed for the UV laser to fire its pulse (the dead-time for the UV laser is 200 μ s). So at high particle concentrations, when one or more particles cross the UV detection zone

during the 200 µs dead-time, the they will not be detected by the photomultiplier tube detector and hence the UV laser counter misses the other particles which already have been counted by the APS (Agranovski et al., 2003c). Further, the particles can also be counted coincidently by the APS part, so that when more than one particle is present in the detection zone (as will often occur with increasing particle concentrations), all of the particles in the detection zone will be counted, however this does not with the UV laser reading (TSI-Incorporated, 2000). This study showed that the maximum detection limit of the fluorescent particles for the UVAPS (the linear region illustrated in Figure 10) is approximately 7×10^7 particles/m³ (for the total particles). In other words, if the fluorescent percentage of the sample is 100%, then the coefficient efficiency of the UVAPS for measuring fluorescent particles will be approximately 7×10^7 particles/m³. This result is consistent with the upper detection limit of the UVAPS for measuring bacteria, which is 6×10^7 particles/m³ (Agranovski et al., 2003a).

On the other hand, the sensitivity of the UVAPS was high with respect to the fungal spores under investigation. The instrument showed a good ability to count and to differentiate *A. niger* and *Penicillium* sp. spores under controlled laboratory conditions. When measuring one spore, the UVAPS locates it in one of the 64 channels: in channel one (if the particle is non-viable or when the content of specific fluorophores is lower than the detection limit of the UVAPS) or in one of the fluorescent channels (2-64), depending on the amount of fluorophores in the particle. While Figure 5c shows fungal spores of *Penicillium* sp. with a concentration of 0.006 particles/cm³, the UVAPS lower detection limit was found to be 0.01 particles/cm³ (as total concentration). Some of the spores are located in the fluorescent channels, with one in channel one. The sensitivity of the UVAPS in measuring fungal spores depends

not only the spores themselves, but also the instrument setting parameters. While the ability of the UVAPS for measuring fungal spores was high, it was limited to measuring only individual bacterial spores (Agranovski et al., 2003) when similar UV laser pulse energy and the PMT gain settings were used ($50 \pm 1\%$ of the laser's full power and 482 V in this study and 50% and 500 V for Agranovski et al., 2003). For instance, in Agranovski et al. (2003) the fluorescent percentage of B. subtilises spores was 1.5 %, which was less than that of background aerosols (1.8 %), compared to 81.5% and 62.4% for Penicillium and A. niger, respectively. Again, this difference may be due to the amount of viable spores for the two microorganisms (fungi and bacteria) under investigation and also may be due to the smaller size of B. subtilises (count median diameter of 1.09 µm) compared to fungal spores.

It has to be, however, noted that the concentration of fungal spores in the environmental samples are typically considerably smaller than what was investigated in this study. For example, (Hargreaves et al., 2003) has reported that the concentrations of airborne fungi in environmental samples measured outdoors and indoors (in 14 residential suburban houses in Brisbane) are 1133 ± 759 CFU/m³ and 810 ± 389 CFU/m³, respectively. The study by Lee et al. (2006) has reported that the concentration of airborne fungi in six Cincinnati homes is typically between 0 and 1362 CFU/m³. As UVAPS lower detection limit was found to be 0.01 particles/cm³, to study fungal aerosols at such concentration levels, the UVAPS has to be operated with a dedicated concentrator. The sensitivity of the instrument in the field requires, however, further investigation.

4. Conclusions

This work has investigated the performance of the UVAPS for monitoring fungal aerosols under controlled laboratory conditions. The instrument was found to be quite sensitive for detecting the fluorescent biomolecules present in the fungal spores investigated. The UVAPS results were comparable with the results of the AGI-30 impinger, which was used as a reference sampler. A linear relationship was observed for the total particles and the fluorescent particle concentrations, measured by the UVAPS within the concentration range up to approximately 7×10^7 particles/m³. This number represents the upper limit of detection of the UVAPS for fungal particles.

The study has also investigated two methods for generating fungal aerosols. While the Collison nebulizer was found to be unable to generate fungal aerosols with diameters of 5 μ m and larger, the dry generation method has proved to be reproducible and easy to control, as well as simple and inexpensive.

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Table Captions

- Table 1. Tested fungi characteristics.
- Table 2. Aerosol results as measured by the UVAPS and the AGI-30.

Figure Captions

- Figure 1. Experimental set-up (Collison nebulizer method).
- Figure 2. Experimental set-up (direct method).
- Figure 3. The UVAPS spectra for blanks and standards: (a) Blank (direct method); (b)
 PSL (0.993 μm) generated by Collison nebulizer; (c) Blue fluorescent (0.91 μm) generated by Collison nebulizer.
- Figure 4. UVAPS spectra (APS part) for aerosols generated by collison nebulizer: (a) (SS-2-PXG 0.1) standard; (b) A mixture of (SS-2-PXG 0.1), (SS-5-PXG 0.1), (SS-7-PXG 0.1), (SS-10-PXG 0.1) and (SS-15-PXG 0.1) standards; (c) *Penicillium* species (ACM4616).
- Figure 5. The UVAPS spectra for fungal spores (direct method): (a) *Penicillium* sp.;(b) *A. niger*; (c) *A. niger* (low concentration).
- Figure 6. The UVAPS spectra (APS part) for fungal aerosol: (a) *Penicillium* sp.; (b)*A. niger;* (c) fragmentation of *Penicillium* sp. at air flow rate of 25 L/min.
- Figure 7. Fungal spore measurements of UVAPS and AGI-30 impingers during the experiment investigating their correlation: (a) *Penicillium* sp.; (b) *A. niger*.
- Figure 8. The range of total and fluorescent particle concentrations from fungi as measured by UVAPS during the experiment investigating the correlation with AGI-30 impingers.
- Figure 9. The range of the total concentrations and fluorescent percentage for high fluorescent percentage cultures as measured by the UVAPS.
- Figure 10. Fluorescent particles of *Penicillium* as a function of total particles measured by the UVAPS.

Table 1. Tested fungi characteristics.

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Fungal	Source	Conidial	Shape	Width, Length	M.A.D ^a
spores		head colour		(µm)	
Penicillium	(ACM 4616)	Greenish blue	Subglobose	e^b 2-4 ^c	2.53 ± 0.10
A. niger	(ATCC 9142)	Brownish black ^d	Globose, subglobose	$2.5-5.0^{d}$	3.51 ± 0.08
a					

^{*a*}Mean Aerodynamic Diameter as measured by UVAPS. ^bAs identified by light microscopy. ^{*c*}(Ramirez, 1982)

^d(Raper et al., 1965)

Aerosol results as measured by UVAPS and impingers.

Aerosol	Aerosol <u>aTotal particles</u>			^b Fluorescent particles			^c Culturing particle	
	Conc.	M.A.D	^e G.S.D	Conc.	M.A.D	F%	Conc.	^f Cul. %
	$\#/m^3$	μm		$\#/m^{3}$	μm		CFU/m ³	
Blank	9.0E+04	0.86	1.29	0.0	0.0	0.0	0.0	0.0
PSL (0.993	3) 1.8E+07	1.00	1.21	2.4E+04	1.40	0.13	-	-
Bf (0.91)	1.6E+07	0.90	1.15	1.6E+07	0.90	97.6	-	-
Penicilliun	n 1.2E+07	2.55	1.28	9.8E+06	2.50 ± 0.11	81.5	8.0+E06	67.3
^g Penicilliu	m3.0E+06	2.66	1.24	7.5E+05	2.63 ± 0.10	25.1	6.6+E05	22.0
A. niger	5.5E+06	3.47	1.31	3.4E+06	3.47 ± 0.09	62.4	2.8+E06	50.5
^h A. niger	1.9E+06	3.54	1.26	2.9E+05	3.53 ± 0.07	15.3	3.2+E05	16.8

^aTotal particles are particles counted through the channels 1-64.

^bFluorescent particles are counted through the channels 2-64.

^cCulturable particle are particles measured by the AGI-30 impingers.

^dM.A.D is the mean aerodynamic diameter.

^eG.S.D is the geometric standard deviation.

^fCul.% is the percentage of culturable particles.

^g*Penicillium* sp. with low fluorescence culture.

^hA. *niger* with low fluorescence culture.



- Fig. 1. Experiment set-up (Collison nebulizer method). ^aFlow Meter ^bDryer
- (c) Pressure equalizing holes



Fig. 2. Experimental set-up (direct method) (c) Pressure equalizing holes





Fig. 3. UVAPS spectra for blank and standards: (a) Blank (direct method); (b) PSL (0.993 μ m by Collison nebulizer); (C) Blue fluorescent (0.91 μ m by Collison nebulizer).



(c) Penicillium species

Fig. 4. UVAPS spectra (APS part) for aerosols generated by collison nebulizer: (a) (SS-2-PXG 0.1) standard; (b) A mixture of (SS-2-PXG 0.1), (SS-5-PXG 0.1), (SS-7-PXG 0.1), (SS-10-PXG 0.1) and (SS-15-PXG 0.1) standards; (c) *Penicillium* species (ACM4616).



(a) Penicillium sp.



(b) A. niger



(c) Penicillium sp.

Fig. 5. UVAPS spectra for fungal spores (direct method): (a) *Penicillium* sp.; (b) *A. niger*; (c) *Penicillium* sp. (low concentration).



Figure 6. UVAPS spectra (APS data) for fungal aerosols: (a) *Penicillium* sp. with out fragmentation; (b) *A. niger*; (c) Fragmentation of *Penicillium* sp. at air flow rate of 25 L/min.



(b)

Fig. 7. Fungal spore measurements of UVAPS and AGI-30 impingers during the experiment investigating their correlation: (a) *Penicillium* sp.; (b) *A. niger*.



Fig. 8. The range of total and fluorescent particle concentrations from fungi as measured by UVAPS during the experiment investigating the correlation with AGI-30 impingers.



Fig. 9. The range of total concentrations and fluorescent percentage for fungi (of high fluorescent percentage), as measured by UVAPS, during its correlation with AGI-30 impinger.



Fig. 10. Fluorescent particles of *Penicillium* sp. as a function of total particles by UVAPS, similar trend was obtained for *A. niger* (data not shown).