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PAPER

Comparison of three high-flow single-stage impaction-based air samplers for bacteria quantification: DUO SAS SUPER 360, SAMPL'AIR and SPIN AIR†

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Indoor Air Quality (IAQ) can be significantly deteriorated by high levels of bioaerosols that may cause adverse health effects in building occupants. There is no standard method for the quantification of this kind of pollutants and several protocols and sampling devices are used. The aim of this work was to compare three commonly used portable air samplers available in the market. DUO SAS SUPER 360, SAMPL'AIR and SPIN AIR units were tested simultaneously for bacteria quantification in a laboratory room in realistic conditions. The results obtained showed that the SPIN AIR unit was the most precise and recovered a higher amount of colony-forming units; consequently this sampler seems to be better for indoor-air bioaerosol concentration measurements. Additionally, positive-hole correction can be avoided due to the SPIN AIR sampling head rotation mechanism. The mean bacterial concentration measured by the other two models was not significantly different. However, due to the high dispersion of the DUO SAS SUPER 360 results, many repetitions are necessary to obtain a reliable measure with this device.

1. Introduction

The concern about indoor air quality has grown in tandem with the recognition of building-related illnesses: allergies, asthma, sick building syndrome, *etc.* Therefore, controlling the microbiological quality of the air is essential not only in pharmaceutical manufacturing areas, hospitals, food processing facilities, *etc.* but also in homes and workplaces.[1] As a result, there is a need for systematic control of air pollution, both chemical and biological. Nevertheless, there is still a lack of regulation of acceptable concentration levels for microorganisms and standard methods for estimation of this concentration.

The first step in the assessment of biological contamination indoors is a thorough inspection by an experienced investigator followed by the quantification and identification of the microorganisms present in the air. To that end, several air sampling devices have been widely employed.[2] Although there is plenty of literature on sampling methodology, such studies do not conclude any standard device or method of sampling for the quantification of microorganisms. Thus, results obtained by different researchers at different places cannot be compared. Consequently, there is a need of well characterized samplers for microbiological aerosols and comparisons between them.

Bioaerosols are collected by separating the particles from the air stream using different physical forces. These forces constitute a base for classification of air samplers in inertial and non-inertial devices.[3] The accuracy in the measurement of air microbial contamination is dependent on obtaining representative samples from the air and limited by the errors of the sampler performance. Frequently, microorganisms are not found as single cells in the air, but tend to form aggregates (clustered to each other) or attach to abiotic particles.

Inertial samplers include impactors, impingers and centrifugal samplers. In centrifugal samplers the air is forced into a centrifugal motion and the particles are deposited on the sampler wall (wet or dry). In the collection stage of impactors and impingers the air stream is forced in one direction where particles are impacted on a solid or liquid surface, respectively. Cascade impactors include several collection stages that give information on the aerosol size distribution. In multi-hole impactors the particles are collected on a standard size Petri dish containing

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nutrient medium. Subsequently, the agar-filled Petri dishes are incubated for the growth of fungal or bacterial colonies.[4] Despite the advantages, some drawbacks of this kind of samplers are: the rapid overloading of the plates when the rooms are not so clean; the problematic quantification of non-culturable microorganisms, which may be harmful as well; agglomeration of microorganisms, stress of impaction and electrostatic attraction of particles to the plastic agar plates.[5]

Non-inertial samplers include electrostatic and thermal precipitators and filters. Filtration is simple and relatively inexpensive but it poses two major disadvantages: dehydration that may be caused by large air volumes passing over a bioparticle after its deposition on the dry collection medium and the recovery of the deposited material from the filters. In the precipitation method, the particles are separated from the air stream by electrical forces or a temperature gradient, indeed collection is achieved with little pressure drop and a relatively small amount of power is needed. Nevertheless, electrostatic precipitators produce ozone and nitrogen oxides and thermal precipitators have a small collection area compared to other samplers. Furthermore, the temperatures generated may adversely affect the culturability of some microorganisms. Consequently, they have been mostly a research tool and have little serious application for bioaerosol collection.[6]

Other bioaerosols collection methods such as gravitational settling on agar surfaces (sedimentation plates) are not quantitative, since the number of microorganisms cannot be related to any specific air volume.

Taking into account all these factors, the impaction-based instruments seem to be the most interesting samplers for indoor air. The total efficiency of these samplers is determined by several factors such as the design of the inlet and the collection stage, the flow rate and the choice of collection medium. These factors also affect the viability of the collected microorganisms.[3] Underestimations can occur either due to a failure of the sampling device to capture microorganism-containing particles (physical loss) or due to biological losses, such as the inactivation of culturable microorganisms during the collection or the inability of some microorganisms to grow in the agar medium selected.

Traditionally, the most widely used air impactor is the Andersen sampler. This device needs to be attached to a sampling pump that works at a flow rate of 28.3 L min^{-1}. The original six-stage cascade impactor was designed to collect bioaerosols that could be directly related to particle deposition in the human respiratory system and allows simultaneous sizing and counting of culturable microorganisms.[7] The Andersen sampler is available with 1, 2, 6 and 8 stages and has been recommended for monitoring airborne microorganisms in office environments.[8]

Nowadays a great number of inertial impaction samplers have been commercialized by different companies, and high-flow portable samplers with an integrated sampling pump have become very popular. In this paper three single-stage inertial impaction samplers commonly used for microbiological sampling are evaluated and compared in side by side tests using natural bioaerosols in a real environment. The impactors selected were DUO SAS SUPER 360 (International PBI, Milan, Italy); SAS sampler is mentioned in Spanish standard UNE 171330-2 as an example of the microbiological air monitoring method and used for air quality control on board of space stations, and two alternatives: SPIN AIR V2 (IUL S.A., Barcelona, Spain) and SAMPL'AIR (AES Chemunex, Bruz, France). All the devices selected have a sieve plate similar to that of the Andersen, but as a difference, they all have an integrated sampling pump fed by an internal battery, which makes them independent of the electrical network. Furthermore, since they are light, silent and easy to carry, they serve as portable multi-hole impactors and are useful for indoor sampling in offices, hospitals, schools and even remote areas. Additionally, the high-flow of these samplers allows the analysis of environments with a minimum level of contamination, such as "clean rooms", or where the sampling of large air volumes in short periods of time is needed. They all permit to record, transfer to a PC or print the sampling information.

Many studies compared the Andersen sampler with other samplers or sampling methods, for instance the All Glass Impinger (AGI),[9,5] the Nuclepore-Filtration–Elution (NFE) method,[5] the May three-stage glass impinger[10] or the Reuter Centrifugal Sampler (RCS).[2] As far as we know, no studies have evaluated or compared the DUO SAS SUPER 360, the SAMPL'AIR or the SPIN AIR V2. There are some studies concerning the single head SAS (Surface Air System) sampler;[9,11] but none on the model with two sampling heads, evaluated in this work.

2. Experimental

The performance of the impactors selected was evaluated by simultaneous indoor air sampling under realistic conditions and subsequent bacterial counting.

2.1. Air samplers

Three single-stage inertial impaction samplers were employed, all of them factory calibrated. The main characteristics of the air samplers compared are presented in Table 1.

(a) DUO SAS SUPER 360 (International PBI, Milan, Italy). The SAS model tested has two independent 219-hole heads for aerosol impaction in the same device but only one aspiration pump that operates at a total flow rate of 360 L min^{-1}, providing an air flow of 180 L min^{-1} per sampling head. The double head allows the operator to do duplicates or to collect two different types of microorganisms at the same time by choosing different culture media.

(b) SPIN AIR V2 (IUL S.A., Barcelona, Spain). This sampler has only one main head, but a slave sampler with its own aspiration pump was attached. The microprocessor and the power supply of the main sampler control the slave. The sampling heads have the possibility of rotation. For comparative purposes, 400-hole sampling heads with 100 L min^{-1} flow rate and 90 mm Petri dishes were selected and the rotation was not activated. In addition, rotation and non-rotation experiments were performed.

(c) SAMPL'AIR (AES Chemunex, Bruz, France). This is a sampler with a single 256-hole impaction head that works at a flow rate of 100 L min^{-1}. Its performance is validated according to ISO0020 14698-1 by the Health Protection Agency (HPA, UK).

Table 1 Main characteristics of the three air samplers studied

	DUO SAS SUPER 360	SAMPL'AIR	SPIN AIR V2
Number of heads	2	1	1 + 1 slave
Air flow/L min^{-1}	360, 180 per head	100	100/Petri, 60/Rodac
Battery autonomy/h	7	4	8 without slave
Weight/kg	1.75	1.8	1.7 main + 1.35 slave
Number of holes	219	256	400
Diameter of holes/mm	1	0.7	0.7
d_{50}/μm	1	0.5	0.88
Plate rotation	No	No	Yes

2.2. Growth medium

Standard 90 mm Petri dishes containing growth medium for bacteria were loaded into the sampler heads. The sampling heads were rinsed in 70% ethyl alcohol before each sampling. Preliminary tests with different culture media showed that nutrient agar allowed the growth of a greater number of different bacterial species. Accordingly, bacterial samples were collected using nutrient agar (3 g beef extract (Conda Laboratories, S.A., Spain), 10 g bacteriological peptone (Conda Laboratories, S.A., Spain), 5 g NaCl (Panreac, Spain) and 15 g of bacteriological agar (Conda Laboratories, S.A., Spain) in 1 L distilled water, pH = 7.2–7.4). This non-selective medium has been extensively used for the routine isolation and cultivation of heterotrophic environmental bacteria.

2.3. Experimental procedure

The experiments were run late in the morning in a laboratory room of 46 m^2, during the normal activities of the 5 occupants of the room. In order to avoid the interference of airflows, windows and door remained closed and Heating, Ventilation and Air Conditioning (HVAC) system operation was stopped. The samplers were placed on a laboratory table in a straight line at a height of 1.4 m above ground, near occupants' breathing zone. There was a distance of 0.5 m one from another in order to avoid turbulent disruption from samplers exhaust, affecting the capture efficiency of the other samplers. Temperature and relative humidity (RH) were continuously monitored using a humidity and temperature transmitter (type HMP237, Vaisala).

Firstly, the samplers were tested in two by two experiments, in order to avoid the interference of the exhausted air of too many simultaneous devices: in experiment A, four sampling units, two of DUO SAS SUPER 360 model (named SAS-1 and SAS-2 from now on) and two connected SPIN AIR V2 units (master and slave, without rotation), were tested simultaneously; in experiment B, the two DUO SAS SUPER 360 units and one of the SAMPL'AIR model were tested. The air was collected in triplicate in each of the three possible positions: on the right, on the left and in the middle of the table. Therefore, in each experiment 9 samples (3 repetitions per position) were taken with each sampling head (18 samples with each instrument, except for the SAMPL'AIR).

Secondly, a new experiment was performed with two SPIN AIR V2 couples (one master unit and one slave unit per couple). One couple sampled without rotation and the other one with rotation at 1 rpm, in order to check the difference between the two sampling procedures. One sampling was performed with each couple in each of the two possible positions, on the left and on the right, and the procedure was repeated 9 times; therefore 18 samples were taken for each sampling head (36 samples with each rotation speed).

The sampling time for each impactor was set according to its air flow rate in order to collect the same air volume in all samplings, 550 L (Table 2). Preliminary tests were performed to select the air volume value that provided good sensitivity avoiding sample overloading (data not shown).

After the air collection, the plates were removed from the sampling heads and incubated at 37 °C for 48 h. The visible colonies formed by culturable organisms were then counted.

Table 2 Sampling conditions selected

	DUO SAS SUPER 360	SAMPL'AIR	SPIN AIR V2
Air flow rate/L min^{-1}	180	100	100
Sampling time/min	3.05	5.5	5.5
Air volume/L	550	550	550

2.4. Data analysis

Positive-hole correction, based on the theory that as the number of particles impacting on a given plate increases, the probability of coimpaction events also increases,[7] was applied to the number of colony counts obtained (n_f). Positive-hole correction was not applied for the SPIN AIR when the rotation was activated.

Colony counts after positive-hole correction (n_c) for a multiple-hole impactor with "h" holes can be calculated from eqn (1):[12]

$$n_c = n_f \left(\frac{1.075}{1.052 - f} \right)^{0.483} \quad \text{for} \quad f < 0.95 \tag{1}$$

where $f = n_f/h$.

The concentration of microorganisms in the air, expressed in CFU m^{-3}, was calculated using eqn (2):

$$[\text{bioaerosol}] = \frac{n_c}{Qt} \tag{2}$$

where Q and t are the flow rate and the sampling time for each sampler, respectively.

The data obtained were statistically analyzed using SPSS for Windows (version 14.0). The Shapiro–Wilk test was done to assess the normal or not normal distribution of data. It allows to test the null hypothesis that a sample comes from a normally distributed

population. Levene's test was used to evaluate the equality of variances in our samples. If the resulting p-value of Levene's test is < 0.05, we can reject the homogeneity of variance. As the sample size for every sampler head was small (9 repetitions) and some sampling heads CFU m^{-3} data did not follow a normal distribution, the Kruskal–Wallis test was used for mean comparisons. When means differed statistically ($p < 0.05$), pair comparisons were done using the Mann–Whitney test to find out which means differed from the others ($p < 0.01$). This test is applied for small sample size ($N < 30$) and no normal distribution.

3. Results

3.1. Experiment A: SPIN AIR V2 – DUO SAS SUPER 360

Both samplers have two heads, which allow not only to compare their results, but also to check their internal performance. Table 3 shows the results of the measurements performed simultaneously with these two types of samplers.

For the same air volume, the bacteria counts were higher and the data deviation lower with the SPIN AIR than with the two DUO SAS SUPER 360. Moreover, the mean concentration obtained in both SPIN AIR sampling heads was similar, 134.7 CFU m^{-3} (main) and 133.3 CFU m^{-3} (slave). Standard deviations and standard errors were around four times higher for DUO SAS samplers than for the SPIN AIR sampler and the mean values obtained with the four SAS sampling heads differ. DUO SAS-2 sampler mean concentration was the lowest, with 82.4 CFU m^{-3} recovered with the left head and 79.2 CFU m^{-3} recovered with the right head.

Fig. 1 shows the box plot for all the sampling heads employed in the experiment A, from which the data dispersion and skewness can be analyzed.

Right and left head distributions were more similar for the SAS-1 sampler than for the SAS-2 sampler. The dispersion of CFU m^{-3} data was greater for the left head for both SAS samplers. Moreover, SAS-2 right head data had an outlier (unusual observation) and a positive skewness. The SPIN AIR data distribution was similar for both heads and the data dispersion was lower than the other samplers. Furthermore, the median values were higher with this sampler.

The Kruskal–Wallis test showed that CFU m^{-3} data of the different sampling heads differed statistically in their means ($p = 0.019$). In order to find out which of the means differed, the Mann–Whitney test was done for every pair of sampling head data. It was found that the right head of the SAS-2 sampler differed statistically in its CFU m^{-3} mean data from SPIN AIR master and slave means ($p = 0.005$).

Table 3 Mean bacteria concentration values obtained simultaneously with two DUO SAS SUPER 360 and one SPIN AIR with a slave

Sampler	Mean, μ/CFU m^{-3}	Standard deviation, σ	Standard error
DUO SAS-1_{left}	114.9	56.4	18.8
DUO SAS-1_{right}	99.5	41.3	13.8
DUO SAS-2_{left}	82.4	49.0	16.3
DUO SAS-2_{right}	79.2	35.4	11.8
SPIN AIR_{master}	134.7	12.3	4.1
SPIN AIR_{slave}	133.3	12.4	4.1

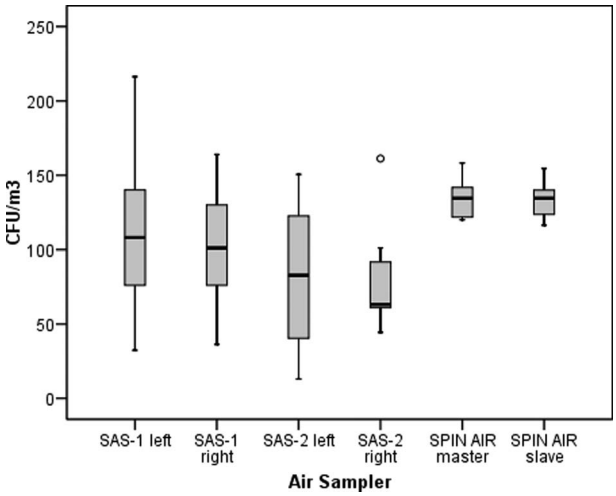

Fig. 1 Box plot of the CFU m^{-3} data obtained in experiment A.

It was observed that the variances of the four SAS sampling head results were homogeneous (Levene's test $p = 0.157$) and, according to the Kruskal–Wallis test for only these four sampler heads ($p = 0.351$), the means cannot be considered statistically different, despite the lower values obtained with the SAS-2 sampling heads.

When the nine values of concentration of bacteria obtained with each of the heads of DUO SAS and SPIN AIR (three repetitions × three positions) are compared, the agreement between SPIN AIR sampling heads can be readily observed (Fig. 2). On the contrary, the values for both DUO SAS samplers are dispersed along (difference among measures of the same magnitude) and at both sides (difference between sampling heads) of the diagonal.

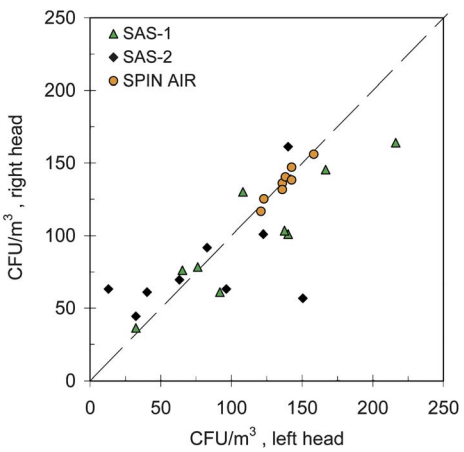

Fig. 2 Identity plot for right and left sampling heads of DUO SAS and SPIN AIR impactors in experiment A.

3.2. Experiment B: SAMPL'AIR – DUO SAS SUPER 360

In experiment B, a double-head sampler was compared with a single-head sampler. Table 4 shows the results of the measurements.

The low significance values are in agreement with the box plot presented in Fig. 3. All sampling heads data, except for SAS-2

Table 4 Mean bacteria concentration values simultaneously obtained with two DUO SAS SUPER 360 and one SAMPL'AIR

Sampler	Mean, μ /CFU m^{-3}	Standard deviation, σ	Standard error
DUO SAS-1$_{left}$	70.4	59.0	19.6
DUO SAS-1$_{right}$	59.5	38.1	12.7
DUO SAS-2$_{left}$	55.1	18.2	6.1
DUO SAS-2$_{right}$	55.7	30.1	10.0
SAMPL'AIR	54.5	16.1	5.4

right, had outliers. Besides, SAS-1 and SAMPL'AIR data distributions were positively skewed. However, the median values were very close.

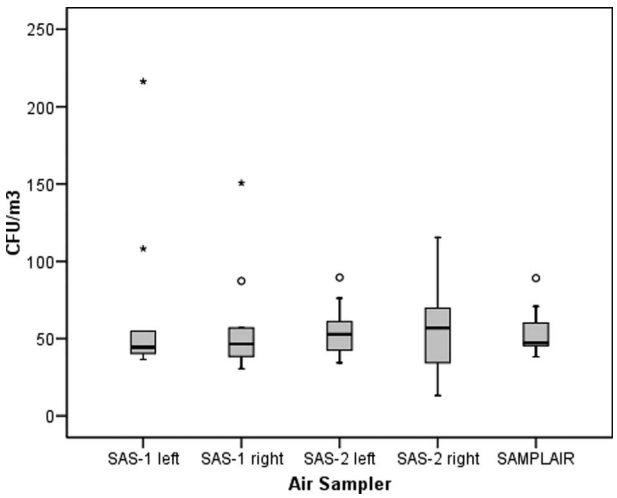

Fig. 3 Box plot of the CFU m^{-3} data obtained in experiment B.

The Kruskal–Wallis test applied to CFU m^{-3} data from all the sampling heads showed that they did not differ statistically in their means ($p = 0.989$). The media obtained cannot be considered significantly different despite the high number of CFU m^{-3} recovered by the left head of DUO SAS-1 sampler, 70.4 CFU m^{-3}. The difference between the sampling heads operation of the DUO SAS samplers can be clearly observed in Fig. 4.

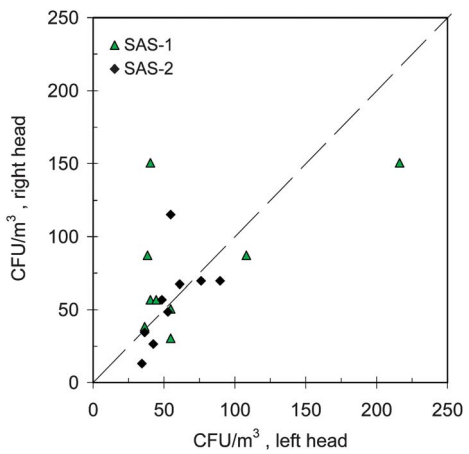

Fig. 4 Identity plot for right and left sampling heads of DUO SAS impactors in experiment B.

3.3. SPIN AIR V2: rotation *vs.* no rotation

The colony counts (n_f) per m^3 obtained with plate rotation at 1 rpm (mean: 19.3, standard deviation: 5.0) were comparable to those obtained without rotation after positive-hole correction (n_c) (mean: 19.9, standard deviation: 7.1) ($p = 0.849$). The standard deviations were similar as well.

4. Discussion

Fig. 5 shows the average bacterial concentration obtained from both DUO SAS sampling heads for the three tested positions, compared to the mean concentrations of the two other samplers, SPIN AIR (experiment A) and SAMPL'AIR (experiment B). As it was statistically determined, similar values were obtained with DUO SAS and SAMPL'AIR and slightly different with DUO SAS and SPIN AIR. SPIN AIR mean concentration of bacteria recovered remained stable in the three positions, whereas DUO SAS values were variable and always lower than SPIN AIR. Very little dispersion was observed in SPIN AIR data compared with the high dispersion given by the SAS in the same experiments.

Fig. 6 combines the results of experiments A and B; the global average concentration of bacteria recovered for each sampler model is represented. These results are the mean of the 18 replicates made with SPIN AIR and DUO SAS and 9 replicates made with the single-head SAMPL'AIR.

The highest number of CFU m^{-3} was achieved with the SPIN AIR sampler; at the same time the error and the coefficient of variation (CV) were the lowest. In contrast, the SAS sampler presented the highest CV. Since it is a high-flow sampler and the sampling heads are sharing the aspiration pump, an unequal flow distribution between the sampling heads might have favored this variation. Although this configuration makes the instrument very easy to handle, it is difficult to assure a perfect division of one air stream into two streams of exactly the same flow rate. This problem was solved in the SPIN AIR V2 sampler, where the slave head, despite being controlled by the main one, includes its own aspiration pump. Additionally, fluctuating sampler characteristics have been previously found for other single-head SAS models,[13] which may contribute to a higher CV for this sampler, and therefore lower reproducibility.

In previous building samplings and laboratory experiments the authors have suspected operational problems with the DUO SAS SUPER 360 samplers. The authors had a concern about the accuracy of the results obtained with DUO SAS units because of the high variability observed (data not shown). It was necessary to do a high number of replicates when using these SAS samplers in order to obtain reliable measurements. The results of the present work confirm the variability of these samplers compared to SAMPL'AIR and SPIN AIR; therefore the measures performed with the SAS units should be interpreted with caution. The coefficients of variation obtained were much higher than those of SAMPL'AIR or SPIN AIR and even to some values of CV reported in the literature for the SAS single-head model.[11,14]

According to this work, the SPIN AIR recovered a higher number of CFU, with no difference between main and slave heads and it had the lowest coefficient of variation, therefore it seems to be the most accurate of the three portable impactors evaluated. Moreover, the possibility of rotation allows the use of

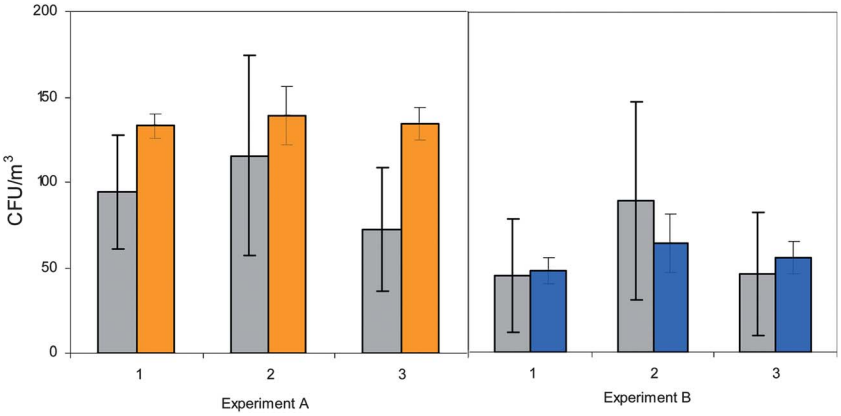

Fig. 5 Bacteria concentration mean values obtained in the three possible positions during the experiments: (A) DUO SAS (left, grey) *vs.* SPIN AIR (right, orange), (B) DUO SAS (left, grey) *vs.* SAMPL'AIR (right, blue).

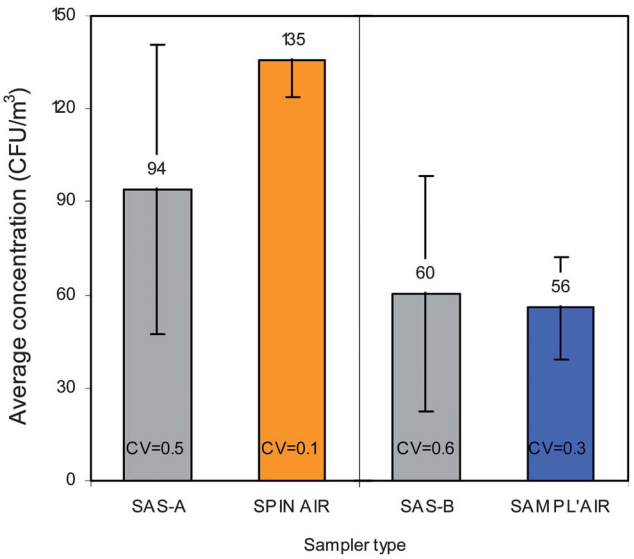

Fig. 6 Bacteria concentration mean values obtained with the three sampler models in all experiments and coefficient of variation.

a larger portion of the plate surface and therefore positive-hole correction can be avoided. It has been reported that this correction emphasizes the differences between samples, decreasing the reproducibility.[13]

The higher CFU collected with SPIN AIR could be due to the higher number of holes of the sampling heads. In previous studies with other air samplers (Andersen model), it was suggested that it is advisable to use impactors with the greatest number of sampling holes because this decreases the likelihood that multiple particles are deposited at the same impaction sites.[17] However, due to the limitations of this study, it must be taken into account that different results could be obtained in different environmental conditions or with a different protocol.

There is some agreement in the literature regarding underestimation of CFU counts by a single-head SAS sampler.[11,15,16] For example, Bellin and Schillinger reported that on five occasions throughout the year a single-head SAS sampler recovered consistently lower levels of airborne fungal propagules than the Andersen N6 single-stage impactor.[11] However, in most of the samplings the underestimation was found to be not statistically significant. The results presented in this paper indicate that the double-head SAS model seems to underestimate the bacteria concentration relative to that shown with the SPIN AIR. The higher median aerodynamic diameter or "cut off size" of the DUO SAS sampler, that is, the particle diameter at which the sampler has an efficiency of 50%, could be one of the causes.

The concentration levels found with SAMPL'AIR were similar to DUO SAS, despite the lower d_{50} of this model (0.5 μm). A remarkable disadvantage of the former is the single sampling head, due to the impossibility of collecting simultaneous samples without activating multiple instruments. On the contrary, this sampler is the easiest to use, especially in terms of loading and unloading sampling media.

As an additional comment on the evaluation of these devices, some experimental difficulties related to failures on the mechanical performance of the samplers should be pointed out, for instance: the battery autonomy was lower than specified, sometimes flow errors were reported during the measurements and flow rate calibration had to be checked, since some irregularities were detected.

5. Conclusions

In order to standardize indoor-air sampling methods for airborne microorganisms the performance of the sampling devices must be characterized. In this article three portable high-flow single-stage impaction-based air samplers were compared for bacteria sampling. The mean concentrations measured by SAMPL'AIR (from AES Chemunex) and DUO SAS SUPER 360 (from PBI International) were similar, but the latter requires many repetitions due to the high data dispersion between heads and among consecutive measures. SPIN AIR V2 (from IUL) gave the highest concentration with the lowest data dispersion and therefore was the most precise.

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