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Measurements of the medium and low concentration levels of airborne bacteria and fungi by using the new bioaerosol sampler

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ABSTRACT

The sampling process of bacterial and fungal aerosol particles is discussed indicating that a new bioaerosol sampler for extremely low concentration level of these bioaerosols is needed. In this paper the results of the pilot study of the prototype of this new sampler are presented. During these field studies the concentration levels of airborne bacteria and fungi were obtained using simultaneously the new sampler and the reference cascade Andersen 6-stage impactor, as well as the Air Ideal sampler for bioaerosols. The measurements indicated that the assumed designing parameters for the new sampler are suitable and guarantee high collection efficiency of this instrument.

1. INTRODUCTION AND SAMPLING TIME CONSIDERATION

Certain human pathogens seem to be significant causes of infection from indoor air, infecting otherwise healthy individuals (Wanner et al. 1993; Burge 1995; Ross et al. 2000; Turbeville et al. 2006; Jie 2012). Exposure to bioaerosols should be controlled in such indoor environments like kindergartens, elementary schools (Kim et al. 2007) and others, even in underground railway stations (Hwang et al. 2010).

On the other hands, airborne bacteria and fungi may be especially hazardous in clinics and hospitals where they may be the major factor in the increasing morbidity from respiratory diseases (Schaal 1991; Sarica et al. 2002). Some bacteria such as S*treptococcus pyogenes, Neisseria meningitidis, Corynebacterium diphtheriae* and *Mycobacterium tuberculosis* are known to be transmitted predominantly by airborne droplets from infected people, and they may cause nosocomial infection (Letrilliart et al. 2001). It is interesting that many opportunistic bacteria found in the indoor environment pose a potential threat only to immunocompromised patients in hospitals. Therefore, it is necessary to assess the composition and concentration of airborne microorganisms in clinics and hospital buildings. In particular, bioaerosol monitoring in hospital can provide information for epidemiological investigation of nosocomial infectious diseases, research into airborne microorganism spread and control, monitoring biohazard procedures, and can be used as a quality control measure.

Among the available sampler types, impactors are commonly used because of their ability to collect microorganisms directly onto agar growth medium without the need for post collection sample processing (Lin and Li 1999; Reponen et al. 1998). The collection characteristics of impactors depend on their design parameters, such as nozzle diameter, ratio of the jet-to-plate distance over the nozzle diameter (*S/W)* and sampling flow rate.

After impaction, the number of particles collected, *N*, can be expressed by the average concentration, C_a, times the sampled volume, *V*, or the sampler`s flow rate, *Q*, multiplied by the sampling time, *t* (Nevalainen et al. 1992):

$$N = C_a\, Q\, t \qquad (1)$$

Since it is extremely important to collect the optimal number of bioaerosol particles,

before sampling the optimal sampling time should be estimated from the Eq. (1):

$$t = N/(C_a Q) \quad (2)$$

where C_a is now the expected (assumed) concentration of bioaerosol, while N is the optimal number of collected particles (in our case it is an optimal number of colonies which will grow after viable bioaerosol particles will be collected). For the typical Petri dish N = 50. More generally, it can be written:

$$N = \delta A \quad (3)$$

where δ is the optimal surface density of a sample collected on the surface A.

Therefore, the optimal sampling time depends on the assumed/expected concentration level of airborne bacteria or fungi and on the flow rate being the most important parameter of the used sampler for bioaerosol. However, to collect the optimal number (for example, 50) of colony forming units (CFU) when the expected concentration of bioaerosol is very low, the flow rate or the sampling time should be significantly increased. Unfortunately, increasing the flow rate means the increase of the impaction stress of collected microorganisms. Too high a velocity of collecting particles results in a high shear force which may cause serious damage decreasing their viable recovery. Also the second solution to keep the appropriate value of N leads to the sampling stress, which can result in the loss of culturability of airborne microorganisms. It has been observed that increased sampling time has resulted in decreased viability for aerosolized vegetative bacterial cells. As air flows over the nutrient agar surface of an impactor, the agar may lose water content, resulting in a hard surface.

The above discussion clearly indicates that a new bioaerosol sampler for extremely low concentration level of airborne bacteria and fungi is needed. In this paper the results of the pilot study of the prototype of this new sampler are presented.

2. METHODS

During these field studies carried out in both the office and the sterilized room the concentration levels of airborne bacteria and fungi were obtained using simultaneously the new sampler and the reference cascade Andersen 6-stage impactor, as well as the Air Ideal sampler for bioaerosols. One of the commercial samplers used for comparison is multiorifice cascade impactor: the Andersen VI-Stage Viable Particle Sizing Sampler (Andersen 1958), which is generally accepted as the standard instrument for viable bioaerosol particles. This impactor operates at a sampling flow rate of 28.3 liters/min. Each stage of this sampler contains 400 orifices with diameters ranging from 1.81 mm in the first stage to 0.25 mm in the sixth stage. The corresponding cutoff sizes for the six stages are 7.0, 4.7, 3.3, 2.1, 1.1 and 0.65 μm. The second commercial sampler was one – stage impactor Air Ideal (bioMèrieux, Marcy l'Etoile, France) operates at a sampling flow rate of 100 liters/minutes. The reported cut-size diameter of this instrument is 3 μm.

Because the patenting process is still not finished the description of the newly developed bioaerosol sampler will be presented later.

In all three samplers microorganisms were collected on nutrient media (specific to either fungi or bacteria) in Petri-dishes located on the stage or stages of used impactors. Malt extract agar (MEA 2%) was applied for fungi, with chloramphenicol added to inhibit bacterial growth. Trypcase soy agar (TSA) was used for bacteria, with cycloheximide added to inhibit fungal growth.

3. RESULTS AND DISCUSSIONS

The counts of airborne bacteria and fungi measured at the same location

(mostly in the office room) with three bioaerosol samplers are shown in Tables 1-4.

Table 1. Concentration levels of airborne bacteria obtained in the office room by using the new bioaerosol sampler (NBS), Air Ideal sampler (AI), and the 6-stage Andersen impactor (And).

No.	Concentration of airborne bacteria [CFU/m^3]			(NBS)/(And)	(NBS)/(AI)
	Air Ideal (AI)	Andersen Impactor (And)	New Bioaerosol Sampler (NBA)		
1	1137	1007	1110	1.10	0.98
2	290	286	390	1.36	1.34
3	1317	1374	1380	1.00	1.05
4	1577	1569	1840	1.17	1.17
5	1697	1385	1360	0.98	0.80
6	1410	1278	1300	1.02	0.92
7	1273	1250	1350	1.08	1.06
			AVERAGED	1.10	1.05

Table 2. Concentration levels of airborne fungi obtained in the office room by using the new bioaerosol sampler (NBS), Air Ideal sampler (AI), and the 6-stage Andersen impactor (And).

No.	Concentration of airborne bacteria [CFU/m^3]			(NBS)/(And)	(NBS)/(AI)
	Air Ideal (AI)	Andersen Impactor (And)	New Bioaerosol Sampler (NBA)		
1	480	470	374	0.80	0.79
2	123	162	170	1.05	1.38
3	457	413	370	0.90	0.81
4	637	596	590	0.99	0.93
5	600	505	640	1.27	1.07
6	537	661	560	0.85	1.04
7	413	421	390	0.98	0.94
8	90	97	100	1.03	1.11
			AVERAGED	0.98	1.01

The flow rate of the new sampler was Q = 0.02 m^3/min. Previous measurements carried out for the higher flow rate (about 40 liter/ minutes and higher) showed that the concentration levels obtained by this new instrument were lower compared to the data obtained by the Andersen impactor and Air Ideal sampler.

The actual data (Tables 1-2) indicate that the New Bioaerosol Sampler (NBS) used in the office room provides concentration data comparable with these obtained by using the Andersen impactor and the Air Ideal sampler. It is interesting to note that the bacteria concentration levels are, as a rule, even slightly higher comparing to results obtained by other samplers.

Table 3. Concentration levels of airborne bacteria obtained in the sterilized room by using the new bioaerosol sampler (NBS), Air Ideal sampler (AI), and the 6-stage Andersen impactor (And).

No.	Concentration of airborne bacteria [CFU/m^3]			(NBS)/(And)	(NBS)/(AI)
	Air Ideal (AI)	Andersen Impactor (And)	New Bioaerosol Sampler (NBA)		
1	0	10	10	1.0	---
2	17	24	30	1.25	1.76

Table 4. Concentration levels of airborne fungi obtained in the sterilized room by using the new bioaerosol sampler (NBS), Air Ideal sampler (AI), and the 6-stage Andersen impactor (And).

No.	Concentration of airborne bacteria [CFU/m^3]			(NBS)/(And)	(NBS)/(AI)
	Air Ideal (AI)	Andersen Impactor (And)	New Bioaerosol Sampler (NBA)		
1	0	0	0	--	--
2	3	7	20	2.86	6.67

The results of the concentration level of airborne bacteria and fungi in the sterilized room (Table 3-4) look especially promising, where the data obtained by the use of the new sampler seems to be significantly higher compared to concentration obtained by using Andersen and Air Ideal samplers.

Table 5 shows the viable bacterial genera identified in samples collected by three samplers in the office room.

Table 5. Viable bacterial genera identified in samples collected by three samplers in the office room.

BACTERIA	PERCENTAGE OF SPECIES IN TOTAL BACTERIA CONCENTRATION (%)		
	AIR IDEAL	ANDERSEN IMPACTOR	NEW BIOAEROSOL SAMPLER
Gram-positive cocci, including:	**35.0**	**39.1**	**36.6**
Kocuria rosea	-	4.0	3.3
Micrococcus spp.	-	4.0	3.3
Staphylococcus capitis	7.0	-	-
Staphylococcus chromogenes	-	8.6	3.3
Staphylococcus lentus	-	4.6	-
Staphylococcus sciuri	28.0	17.9	26.7
Nonsporing Gram-positive rods, including:	**35.1**	**41.7**	**50.1**
	12.3	0.7	-
Arthrobacter spp.	22.8	31.1	43.4
Brevibacterium spp.	-	8.6	6.7
Corynebacterium auris	-	1.3	-
Corynebacterium propinquum			
	14.1	**5.9**	**3.3**
Gram-positive rods, family *Bacillacae*, including:	1.8	-	-
Bacillus circulans	-	4.6	-
Bacillus mycoides	12.3	1.3	3.3
Bacillus pumilus			
	12.3	**6.7**	**3.3**
Actinomycetes, including:	8.8	6.0	3.3
Rhodococcus spp.	3.5	0.7	-
Streptomyces spp.			
	3.5	**6.6**	**6.7**
Gram-negative rods, including:	3.5	6.6	6.7
Pseudomonas spp.			
TOTAL	**100.0**	**100.0**	**100.0**

Although the relative concentration of some species like Gram-negative rods obtained by using the new sampler seems to be higher compared to other results further studies are needed for more precise conclusions.

4. CONCLUSIONS

The measurements indicated that the assumed designing parameters for the new sampler are suitable and guarantee high collection efficiency of this instrument but the flow rate should not exceed 20 l/min. The new sampler can be successfully used in the indoor environment with a low level of airborne bacteria and/or fungi.

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