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Individually ventilated cages – Microbiological containment testing

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Introduction

Modern biomedical research projects need "high quality" laboratory animals in order to obtain repeatability and homogeneity of the experimental data and to reduce the number of animals. It is, thus necessary to use animals standardised with respect to genetic, microbiological and pathological conditions. Environmental control, by eliminating the chemical, physical and microbial contaminants, can guarantee more standardised housing conditions and, at the same time, highly reduce the biological risk for the personnel working with the animals in accordance with the European and Italian legislation. Among the physical parameters temperature and humidity have to be considered the most important for their influence on animal behavior and metabolism. The optimal values for temperature range between 20 and 23 °C and for humidity between 40 and 60 %; in fact it has been widely demonstrated that when these two parameters are too high, they enhance the growth of moulds and the life span of microorganisms and that most bacteria and fungi, that colonize man and animals, grow at temperatures between 25 and 40 °C.

Microbiological quality assurance of laboratory animals aims to produce animals that meet requirements for microbiological quality, and to maintain the same quality throughout the experiment. Outbreaks of infectious disease in laboratory animals have to a great extent adversely affected their use in biomedical research. Some microorganisms occurring in laboratory animals can also affect man (zoonosis) and this risk is also present in animals which are used as a source of sera and vaccines for use in man. Due to the introduction of specified pathogen free (SPF) animals outbreaks of infectious diseases have been in part replaced by more subtle microbial interference in the outcome of animal experiments. Plenty of viruses, mycoplasma, bacteria and parasites that may affect biomedical research exist for all laboratory animal species *(Boot, 1996)*.

The Service for Quality and Assurance of Animal Experimentation routinely controls the animal housing environment. For several years it has been focusing its activity on monitoring microbiological conditions and even in the animal housing it has considered the indication of Whittard (Table 1), as acceptable levels of contamination. With regard to this problem we have analyzed the count of environmental microorganisms in the animal facility rooms and the isolating capacity of a ventilated cage system (IVC, Techniplast Gazzada, Varese, Italy) which has been conceived to protect, by filtering the supply and exhaust circulating air, both the housed animals and the working personnel. The experiment has been performed in a conventional animal facility to verify the possibility to use animals in mixed conditions i.e. animals housed in normal conditions and animals housed in IVC.

Materials and Methods

Individually Ventilated Cages (IVC)

The IVC system formed of a rack for 36 cages and was equipped with air supply and exhaust modules that guarantee the air removal.

The air supply and exhaust modules were provided with a ventilation control system, a pre-filter and a HEPA filter. Adjustable dempers were available to control the relative pressure of the air in the supply and exhaust plenums in such a way that the air

Table 1. Acceptable limits of c.f.u./m^2 for critical environments (left) and normal environments (right). (Whittard, 1981)

Classification	**Acceptable Limits. . c.f.u./m³**	**Classification**	**Acceptable Limits. . c.f.u/m³**
Sterile	0	1° degree	100-250
Class I	5	2° Degree	251-500
Class II	15	3° Degree	501-750
Class III	75	4° Degree	751-1000
Class IV	100	5° Degree	1001-1500
		6° Degree	1501-2000
		7° Degree	more than 2000

pressure within the cages could be either negative, equal or positive to that of the room.

Sample Methods

The air was sampled for microbial contamination with two different methods and then the detection capacity of the two techniques was compared:

- Surface Air System (SAS, Pool Bioanalysis Italiana Italy)

The SAS is a sampling system made of an air collection unit, a mounting for plates containing solid culture media and a device that regulates the sampling time and quantity. The SAS can collect up to 180 l/min.

- All Glass Impinger (AGI-30, Ace Glass, Vineland, N.J., USA)

The AGI-30 is a liquid sampling system that conveys the collected air in a liquid medium and it is generally used when there is a high microbial air contamination since the liquid can be diluted before being plated; moreover the stress and the biological damage caused to the microorganisms are very small.

The AGI - 30 is made of a cylindrical unit containing 20 ml of a liquid (in our case deionized water) and of a curved tube that conveys the collected air into the liquid medium. This tube is connected to a suction pump so that it is possible to regulate the flow of collected air. Max collection rate 12.5 l/min.

Preparation of Bacteria Suspension

We used Pseudomonas fluorescens as a standardized contaminant.

This microorganism (that is non-fermenter gram negative rod) was chosen because it is widely present in the environment and because it shows a pathogenic activity only in immunosuppressed patients.

This microorganism was cultured on Triptose Soya Agar (TSA, Unipath S.p.A., Milano, Italy) and after 24 h of incubation at 30°C the colonies were collected and suspended in physiological solution to obtain a concentration of $6x10^8$ bacteria /ml by McFarland Standard (bioMérieux, France).The liquid suspension of bacteria was then nebulized (PHARMO Neb, Mefar, Bovezzo, Italy) in the room.

Experimental design

To evaluate the microbiological containment capacity of the IVC, our study was divided into two parts :

1. IVC in a facility room;
2. IVC in aerosol chamber;

Each part was composed of two phases in which the IVC was under positive and negative pressure. Temperature and humidity were monitored inside the room by a wall thermo-hygrometer (Salmoiraghi, Milano, Italy) and inside the cages by a mobile thermo-hygrometer (mod. JB812, Oregon Scientific, Italy).

Part 1:

in the first part, the IVC rack was located in a room where 100 rats were already present together with their natural microbial flora. The room was approximately 36 m^3 with three cage racks containing 100 rats and it was equipped with a system of air conditioning, artificial lighting and a thermo – hygrometer.

Contamination was measured in the room by SAS and inside the IVC rack by AGI-30.

To carry out the sampling inside the cages self closing air nozzles were placed in 3 cages through a hole made at 2 centimeters from the bottom of the cage. During the sampling the 3 cages were placed in different parts of the rack.

The cages were connected to the AGI through autoclavable flexible hoses.

The air samples were collected at time 0 and after 1h, 2h, 3h, 4h, 24h and 48h.

Part 2:

the experiment was performed in the aerosol chamber where 12.5 ml of bacteria suspension was spread through a nebulizer. The aerosol chamber used in this study belonged to a laboratory animal facility, had a surface of a approximately 48 m^3 and was equipped with a system of air conditioning, artificial lighting and a thermo–hygrometer.

The sampling point was located at a distance of 1.5m from the nebulizer (which was at a height of 1m from the floor) and at a distance of 0.5m one from the other, all on the same level.

The following sampling procedure was followed:

The air samples were collected before nebulization (time 0) to evaluate the contamination, then at 7, 14, 21, 28, 35 minutes during the nebulization and at 60, 120, 180, 240, 300 after nebulization.

The sampling and culture procedures were repeated identically.

Sampling both in part 1 and in part 2 was carried out with IVC under positive and negative pressure.

At the end of the sampling, SAS plates were incubated for 24h at 30° C and bacterial count expressed as colony forming units (c.f.u.) /m^3 was performed at 24h and 48h.

Aliquots of 0.1 ml of liquid collected from the AGI were spread on 5 TSA plates and incubated at 30° for 24h, and then the number of colonies present in each plate was counted.

Results and Discussion

Results are shown in Tables 2 and 3 and Figure l.

The air sampling in our study was carried out by using SAS and AGI systems which are both active volumetric samplers. They were chosen because they could express the microbial concentration as colony forming units over air volume (c.f.u/m^3) and guarantee a better and higher microbial recovery when compared to the non volumetric plate method *(Clough et al.,1995)*, moreover AGI was the only system which could be used to sample the air inside the cages. AGI could be considered as an interactive system of sampling. This means that by using AGI it is possible to monitor a dynamic microbiological condition and, as far as we know, this should be the first time a microenvironment like a cage has been sampled directly inside with respect to the microbiological quality of the air passing through.

During the experiment comparative sampling tests have been performed between AGI and SAS in order to evaluate a possible correlation between the two systems.

Figure 1 confirmed the capacity of the samplers to give sufficiently precise data about the environmental microbial concentration.

The intra-cage sampling confirmed the capacity of the IVC filter system, both in normal housing condition and in the presence of a high number of micro-organisms to protect the inside of the cage from the diffusion of microorganisms coming from

Tables 2: IVC in facility room
Results phase I: IVC in positive pressure

Table 2 a: c.f.u./m^3 in the facility room with animals by SAS, IVC in positive pressure

Minutes	**0'**	**60'**	**120'**	**180'**	**240'**	**24h**	**48h**
	16	10	12	25	1	13	25
	24	13	12	12	7	16	37
	30	12	17	24	13	9	35
Means	**23**	**12**	**14**	**20**	**7**	**13**	**32**
C.F.U./m^3	**200**	**100**	**117**	**175**	**58**	**108**	**283**

Table 2 b: c.f.u./m^3 at the exhaust hole of IVC in positive pressure, by SAS

Minutes	**0'**	**60'**	**120'**	**180'**	**240'**	**24h**	**48h**
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
Means	**0**	**0**	**0**	**0**	**0**	**0**	**0**
C.F.U./m^3	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 2 c: c.f.u./m^3 inside the cages of IVC, in positive pressure, by AGI

Minutes	**0'**	**60'**	**120'**	**180'**	**240'**	**24h**	**48h**
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	1	0	0
Means	**0**	**0**	**0**	**0**	**0.3**	**0**	**0**
C.F.U./m^3	**0**	**0**	**0**	**0**	**6666**	**0**	**0**

Results phase II: in negative pressure

Table 2 d: c.f.u./m^3 by SAS, with IVC in negative pressure

Minutes	**0'**	**60'**	**120'**	**180'**	**240'**	**24h**	**48h**
	61	49	46	40	33	20	15
	23	37	58	37	36	31	21
	56	44	61	29	32	25	18
Means	**47**	**43**	**55**	**35**	**34**	**25**	**18**
C.F.U./m^3	**442**	**400**	**525**	**316**	**308**	**216**	**158**

Table 2 e: c.f.u./m^3 at the exhaust hole of IVC in negative pressure by SAS

Minutes	**0'**	**60'**	**120'**	**180'**	**240'**	**24h**	**48h**
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
Means	**0**	**0**	**0**	**0**	**0**	**0**	**0**
C.F.U./m^3	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 2 f: c.f.u./m^3 inside the cages of IVC in negative pressure by AGI

Minutes	**0'**	**60'**	**120'**	**180'**	**240'**	**24h**	**48**
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
Means	**0**	**0**	**0**	**0**	**0**	**0**	**0**
C.F.U./m^3	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Tables 3: IVC in aerosol chamber
Results phase I: IVC in positive pressure

Table 3a: - c.f.u./m^3 in the aerosol chamber by SAS, with IVC in positive pressure, before and during nebulization

Minutes	**0**	**7'**	**14'**	**21'**	**28'**	**35'**
	62	1080	1448	1296	1512	1856
	55	936	1592	1832	1696	1376
	22	1128	1472	1424	1568	2072
Means	**46**	**1048**	**1504**	**1617**	**1592**	**1678**
C.F.U./m^3	**425**	**52000**	**75000**	**80000**	**79000**	**88000**

Table 3 b: c.f.u./m^3 in the exhaust hole of IVC in positive pressure, by SAS, before and during nebulization

Minutes	**0**	**7'**	**14'**	**21'**	**28'**	**35'**
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
Means	**0**	**0**	**0**	**0**	**0**	**0**
C.F.U./m^3	**0**	**0**	**0**	**0**	**0**	**0**

Table 3 c: c.f.u./m^3 inside the cages of IVC by AGI, before and during nebulization

Minutes	**0**	**7'**	**14'**	**21'**	**28'**	**35'**
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
Means	**0**	**0**	**0**	**0**	**0**	**0**
C.F.U./m^3	**0**	**0**	**0**	**0**	**0**	**0**

Results phase II: IVC in negative pressure

Table 3 d: c.f.u./m^3 in the aerosol chamber by SAS before and during nebulization

Minutes	**0**	**7'**	**14'**	**21'**	**28'**	**35'**
	9	816	980	984	1104	1128
	11	838	868	888	1056	1200
	16	600	960	1032	1008	1280
Means	**12**	**751**	**936**	**968**	**1056**	**1203**
C.F.U./m^3	**100**	**37349**	**46551**	**48148**	**52519**	**59780**

Table 3 e: c.f.u./m^3 at the exhaust hole of IVC in negative pressure by SAS, before and during nebulization

Minutes	**0**	**7'**	**14'**	**21'**	**28'**	**35'**
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
Means	**0**	**0**	**0**	**0**	**0**	**0**
C.F.U./m^3	**0**	**0**	**0**	**0**	**0**	**0**

Table 3 f: c.f.u./m^3 inside of IVC by AGI, before and during nebulization

Minutes	**0**	**7'**	**14'**	**21'**	**28'**	**35'**
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
Means	**0**	**0**	**0**	**0**	**0**	**0**
C.F.U./m^3	**0**	**0**	**0**	**0**	**0**	**0**

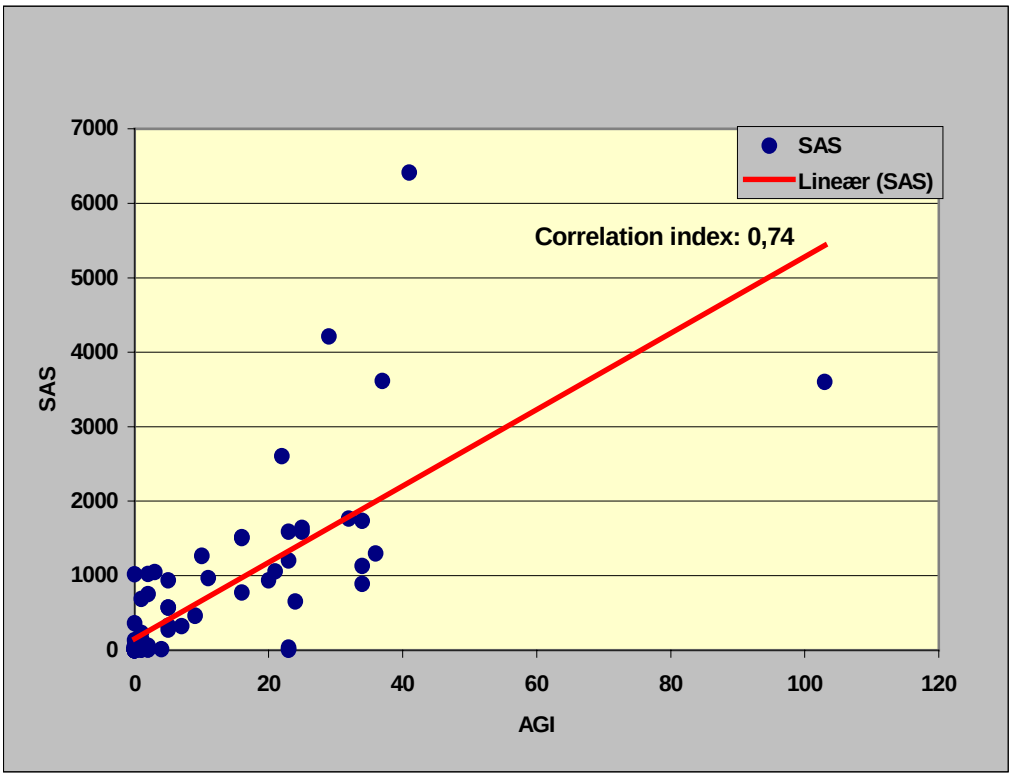

Figure 1. Correlation between AGI and SAS sampling techniques

the external environment and even at the highest microbial concentration the cages were demonstrated to be perfectly sterile inside. These results are furthermore validated by the fact that the air inside the cages was sampled by AGI which is far more sensitive than SAS.

In consideration of the influence of humidity and temperature on bacterial growth, these two parameters were recorded both inside the cages and in the rooms. IVC was shown to be able to maintain humidity and temperature inside the cages uniform and independent of the external environmental fluctuations.

Besides the marked isolation capacity demonstrated by the IVC and the direct advantages coming from the absolute containment demonstrated in negative and positive pressure, the IVC and the AGI sampling methods offer the possibility of investigating the micro-environmental influence related to the single animal and to evaluate the microbiological impact in terms of ratio mass/ volume/c.f.u .

The further objective could be to house different strains of animals in the single cages and to verify the capacity of IVC to protect the animals from environmental microbial infections.

Summary
The aim of our experimental activity was to evaluate the isolating and protective capacity of a ventilated cage system (IVC). IVC can be used to house infected animals and exhaust air, if not perfectly filtered, may represent a potential danger for personnel and other animals housed in the facility. Sampling was performed in a conventional animal facility by SAS and AGI-30 and inside the cages by AGI-30. Our results confirm the capacity of IVC to contain the micro-organisms in negative and positive pressure and demonstrate the ability of AGI-30 to investigate the micro-environmental in the cage.

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