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Research Protocol

Biological Efficiency Evaluation of Active Microbial Air Samplers According to the Simplified Laboratory Method as for EN 17141:2020

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Abstract

The new EN 17141:2020 - Cleanroom and associated controlled environments - Bio contamination control describes an innovative simplified laboratory method for the qualification of microbial air samplers. The purpose of this paper was to demonstrate the practical application of the reported method and to validate the innovative air sampler: TRIO.BAS from Orum International, Italy.

Briefly, as described in Annex E of EN 17141:2020, at point E.6.2 a simplified laboratory method was applied, which consisted of at least two different environments/rooms with an unknown and normally present microbial concentration inside.

The preliminary experiment was conducted within five identified rooms (n = 5) 20m^2 each, in the Department of Veterinary Medicine and Animal Sciences (DIVAS) of the University of Milan, where a previously qualified air sampler: MAS100 from Millipore, USA, the gold standard, was compared with a new instrument to be qualified: TRIO.BAS.

Preliminary test results showed a good efficiency of TRIO.BAS MONO in sampling the airborne microbial concentration.

Keywords: *Air Sampler; Biological Collection Efficiency; Bio Aerosol; Validation; Official Standard Method*

Introduction

The document EN 17141:2020 Cleanrooms and associated controlled environments - Bio contamination control, introduced in August 2020, is the new European standard that replaces the document EN ISO 14698-1:2003 and EN ISO 14698-2:2003 [1,2].

This new standard covers best practices for controlling bio contamination within cleanrooms, controlled environments, clean zones, areas, and spaces. Additionally, information is included on the sources of microbiological contamination, what to include in the microbiological contamination control program, establishing alert and action levels, and information on sampling methods, equipment, and culture media.

According to EN 17141:2020, the supplier of microbial air samplers must demonstrate the collection efficiency by applying the methods listed in this standard.

In particular, in Annex E, under E.6.2, a simplified laboratory method can be applied to validate an instrument.

The method consists of using the laboratory method as an alternative to the aerosol chamber method.

The laboratory method involves the use of generic environments, which will therefore have a typical airborne microbial concentration.

The advantage of this validation test is that it is performed with naturally occurring microorganisms and not with artificial aerosols. Additionally, this test is an easily applicable and a more practical experiment that is equally reliable.

The tests should be performed in at least two different environments/rooms with sufficiently high microbiological concentrations in the air (above 80 CFU/m^3) to ensure that the corresponding number of CFU captured on the culture medium is between 80 and 150 CFU/m^3. Prior to sampling, it may be necessary to adjust the duration of air sampling by the air sampling instrument so that the required number of colonies captured is within the range mentioned above; the sampling time then remains constant for all subsequent tests. The instrument to be validated must be compared with an instrument already validated according to EN 17141:2020.

To this end, the following preliminary study aimed to test the TRIO.BAS MONO instrument (Orum International, Italy) in several rooms of the Department of Veterinary Medicine and Animal Sciences (DIVAS) of the University of Milan, in order to validate it. The gold standard was the MAS100 instrument (Millipore, USA).

Materials and Methods

Experimental plan and air samplers

The preliminary study was set up in Five rooms in the Department of Veterinary Medicine and Animal Sciences (DIVAS).

The TRIO.BAS MONO and the MAS100 (Figure 1), were placed in five different rooms of 20m^2 each at a distance of 1 meter and 80 cm from the floor (Figure 2).

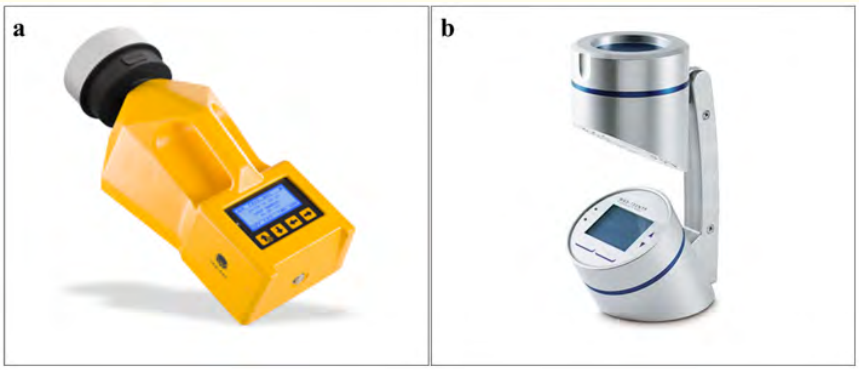

Figure 1: *a) TRIO.BAS MONO (Orum International, Italy); b) MAS100 (Millipore, USA).*

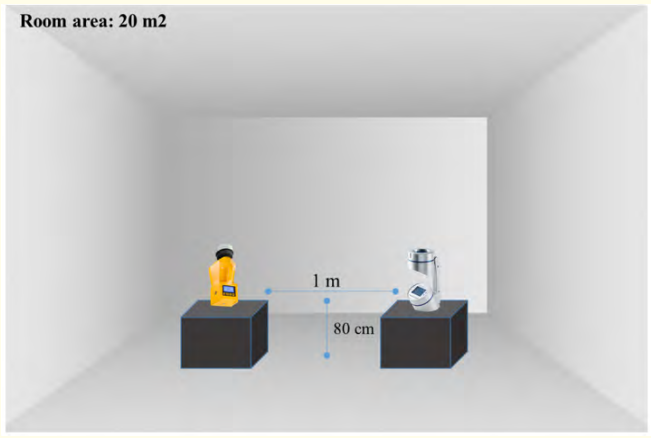

Figure 2: *Graphical representation of the experimental plan.*

Both instruments were set to sample the same volume and flow (100 l/minute).

For the air sampling measurements, the air samplers were powered on at the same time and sampling was carried out on the same day but at different times (morning=first test phase and afternoon=second test phase), the sampling time was set for 10 minutes, the measurement was repeated three times (in triplicate) and the air samplers alternated positions after each sampling.

As this was a preliminary study, the sample size was limited; to obtain a robust and statistically significant validation, the number of samplings will be increased in future experiments.

Subsequently, the following formula was applied to calculate the biological efficiency of the sampler following EN 17141:2020:

(E.1)

Acceptable limits are: (100 ± 50) %.

Microorganisms

Total bacterial counts were enumerated from the air, using ready-to-use petri dishes (90 mm) of Tryptic Soy Agar (TSA; BD BBL™, USA). After sampling, the plates were incubated at 30°C for 48 hours.

Analyses were performed in triplicate and results are expressed as CFU/m^3.

Results

Below were the results of the air sampling which occurred in room 1 (Table 1 and 2), room 2 (Table 3 and 4), room 3 (Table 5 and 6), room 4 (Table 7 and 8) and finally in room 5 (Table 9 and 10).

FIRST test phase						
Environment: Room 1						
TRIO.BAS MONO				MAS100		
Time	Plate 1	Plate 2	Plate 3	Plate 1	Plate 2	Plate 3
09:00-09:10-	210			300		
09:10-09:20		212			250	
09:20-09:30			198			255
	"A" Average CFU/m^3=207			"B" Average CFU/m^3=268		

Table 1: *First day of testing, results of the first test phase in room 1.*

SECOND test phase						
Environment: Room 1						
MAS100				TRIO.BAS MONO		
Time	Plate 1	Plate 2	Plate 3	Plate 1	Plate 2	Plate 3
13:00-13:10-	297			266		
13:10-13:20		280			301	
13:20-13:30			267			248
	"A" Average CFU/m^3=281			"B" Average CFU/m^3=272		

Table 2: *First day of testing, results of the second test phase in room 1.*

FIRST test phase						
Environment: Room 2						
TRIO.BAS MONO				MAS100		
Time	Plate 1	Plate 2	Plate 3	Plate 1	Plate 2	Plate 3
09:00-09:10-	49			89		
09:10-09:20		66			46	
09:20-09:30			64			58
	"A" Average CFU/m^3=60			"B" Average CFU/m^3=64		

Table 3: *Second day of testing, results of the first test phase in room 2.*

SECOND test phase						
Environment: Room 2						
MAS100				TRIO.BAS MONO		
Time	Plate 1	Plate 2	Plate 3	Plate 1	Plate 2	Plate 3
13:00-13:10-	84			140		
13:10-13:20		14			58	
13:20-13:30			47			116
	"A" Average CFU/m^3=48			"B" Average CFU/m^3=105		

Table 4: *Second day of testing, results of the second test phase in room 2.*

FIRST test phase						
Environment: Room 3						
TRIO.BAS MONO				MAS100		
Time	Plate 1	Plate 2	Plate 3	Plate 1	Plate 2	Plate 3
09:00-09:10	106			110		
09:10-09:20		52			74	
09:20-09:30			81			81
	"A" Average CFU/m^3=80			"B" Average CFU/m^3=88		

Table 5: *Third day of testing, results of the first test phase in room 3.*

SECOND test phase						
Environment: Room 3						
MAS100				TRIO.BAS MONO		
Time	Plate 1	Plate 2	Plate 3	Plate 1	Plate 2	Plate 3
13:00-13:10-	25			21		
13:10-13:20		75			58	
13:20-13:30			176			99
	"A" Average CFU/m^3=92			"B" Average CFU/m^3=59		

Table 6: *Third day of testing, results of the second test phase in room 3.*

FIRST test phase						
Environment: Room 4						
MAS100				TRIO.BAS MONO		
Time	Plate 1	Plate 2	Plate 3	Plate 1	Plate 2	Plate 3
09:00 - 09:10	165			111		
09:10 - 09:20		144			111	
09:20- 09:30			194			70
	"A" Average CFU/m^3=168			"B" Average CFU/m^3=97		

Table 7: *Fourth day of testing, results of the First test phase in room 4.*

SECOND test phase						
Environment: Room 4						
MAS100				TRIO.BAS MONO		
Time	Plate 1	Plate 2	Plate 3	Plate 1	Plate 2	Plate 3
13:00-13:10-	104			116		
13:10-13:20		105			103	
13:20-13:30			97			152
	"A" Average CFU/m^3=102			"B" Average CFU/m^3=124		

Table 8: *Fourth day of testing, results of the second test phase in room 4.*

FIRST test phase						
Environment: Room 5						
MAS100				TRIO.BAS MONO		
Time	Plate 1	Plate 2	Plate 3	Plate 1	Plate 2	Plate 3
09:00 - 09:10	67			109		
09:10 -09:20		101			101	
09:20 – 09:30			120			60
	"A" Average CFU/m^3=96			"B" Average CFU/m^3=90		

Table 9: *Fifth day of testing, results of the first test phase in room 5.*

SECOND test phase						
Environment: Room 5						
MAS100				TRIO.BAS MONO		
Time	Plate 1	Plate 2	Plate 3	Plate 1	Plate 2	Plate 3
13:00-13:10-	96			48		
13:10-13:20		54			46	
13:20-13:30			37			100
	"A" Average CFU/m^3=62			"B" Average CFU/m^3=65		

Table 10: *Fifth day of testing, results of the second test phase in room 5.*

Conclusion

The formula for calculating the Biological Efficiency (E.1) of the TRIO.BAS MONO sampler was applied to the results obtained.

The table 11 reported the Biological Efficiency (%) of each day of testing.

Testing days	Biological Efficiency	
First day of testing	%	100,87
Second day of testing	%	101,46
Third day of testing	%	100,77
Fourth day of testing	%	100,82
Fifth day of testing	%	100,98
	Average of biological efficiency	100,98%

Table 11: *Percentage of biological efficiency on each day of testing.*

At the end of the tests carried out in the rooms, and from the calculation obtained with the application of the formula indicated in Chapter E.6.4 of the EN 17141:2020 standard, it was possible to highlight that the air sampler TRIO.BAS, as far as biological efficiency is concerned, has a good ability to collect particles carrying viable microorganisms. in fact, the value of acceptability is included in the limit of acceptability of (100% +/- 50%) foreseen by the standard [3,4].

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