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EC MICROBIOLOGY
Research Protocol

Validation Test of the Collection Efficacy of Airborne Microorganisms by Sampler According to EN 17141:2020 Standard

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Abstract

ORUM international, which has designed and proposes a specific impact instrument such as the TRIO.BAS, has decided to assess the efficiency of the sampler in recovering microorganisms in the air, to carry out the verification microbiological tests indicated in paragraph E.6.2.2 of the EN 17141:2020 standard.

The "E.6.2 Simplified laboratory method" presented in the EN 17141:2020 standard is an alternative to the aerosol chamber method. The advantage is that the simplified method is performed with naturally microorganisms and not with artificial bio-aerosol. In fact, it could be carried out in premises with different levels of airborne contamination. The simplified method is compared to a qualified reference method like the "membrane filter".

The TRIO.BAS microbial air sampler (active sampling) to be qualified was compared with the MD8 Sartorius microbial air sampler (membrane filter sampling) that is a qualified method.

At the end of the test, the results obtained, shown in the tables 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 the following: The air sampler TRIO.BAS, as far as biological! efficiency is concerned, while taking into account the losses caused both by the physical collection efficiency and by the effect that sampling has on the vitality of the microorganisms, has a good ability to collect particles carrying viable microorganisms: in fact, the found value of acceptability is included in the limit of acceptability of (100% +/- 50%) foreseen by the standard.

Keywords: *Airborne Microorganisms; EN 17141:2020 Standard; TRIO.BAS*

Introduction

Air sampler technology currently makes it possible to precisely and timely verify the contamination present in the air by microorganisms.

In the recent standard EN 17141:2020 Cleanrooms and Associated Controlled Environments - Contra i of Biocontamination, in Annex E, the verification of volumetric air samplers used for this purpose is addressed.

ORUM international, which has designed and proposes a particular impact instrument such as the TRIO.BAS, has decided to assess the efficiency of the sampler in recovering microorganisms in the air, to carry out the verification microbiological tests indicated in paragraph E.6.2.2 of the same standard.

Purpose of the Study

The tests described below were set up and carried out with the TRIO-BAS instrument to validate its biological efficiency, that is, its ability to collect particles carrying viable microorganisms, including losses caused by both the efficiency of physical collection, and the effect that sampling has on the viability of microorganisms.

Figure 1: *TRIO.BAS mono microbial air sampler.*

Materials and Methods

The method described in Chapter E.6.2 of EN 17141:2020 was chosen for testing, as an alternative to the planned method of using a chamber containing an aerosol with known titre microorganisms.

The advantage of this method is that uses naturally occurring microorganisms in the air.

To obtain meaningful results, the tests were repeated simultaneously with an already qualified reference instrument using filter membranes. The tests were performed at two different locations, with sufficiently high conventions of microorganisms to give, when the colonies were counted in a Petri dish, a separation between colonies that would ensure easy reading of the results.

Instruments used for the tests

- Microbiological air sampler TRIO.BA$ Serial No. 4621004 (flow rate 100 liters/minute) to be qualifieddirectly impacted on Petri dishes containing agar (calibration certificate attached).
- MD8 Airport Sartorius (16757) Serial number 43303906 (flow rate 50 liters/minute) qualified with impact on filter membrane on Petri plate (calibration certificate attached).

The volumes of air withdrawn from each instrument were related to the sampling times, to be able to express the results in cfu/m^3.

Figure 2

Culture media used for tests

Culture medium used for the TRIO.BAS sampler: BO BBL IC-XT Pack Trypticase Soy Agar w/Lecithin and Polysorbate 80 (30 ml) agar medium in 90 mm LL plates Lot 257729.

Figure 3

Culture medium used for the MD8 sampler: Gelatine Disposable 3 um - Sartorius Stedim - Lot 17528180198.

Figure 4

Count of microorganisms collected during each test

For the counting of the microorganisms collected during each test, the ACCREDIA UNI EN ISO 4833 _2:2013/ECl:2014 accredited analytical method was used (indicated in the attached test reports).

Performance of tests

The tests took place over the course of a day in two different areas of a warehouse, recovering the microorganisms naturally present in 1000 liters of ambient air.

The technicians who performed the tests used protective clothing consisting of overalls, disposable gowns, FFP2 mask, sterile gloves, hat, overshoes.

The air samplings were always carried out with the two instruments positioned to avoid the production of turbulence at the time of each test, under different conditions: for the first test, air was taken from an area with natural movement (in proximity of the open warehouse door and movement of personnel) for the second test the air was taken from an area of the warehouse with shelves and static air. At the end of the two series of tests, the Petri dishes and the gelatin filters used for taking the air were incubated at the temperatures and times foreseen by the accredited test method used for counting the colonies. The results obtained were evaluated keeping in mind the different types of agar media used for the capture of microorganisms from the air to ensure consistent results.

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Results

The analytical result s of the two tests are shown below:

Results tables

First Test						
Environment: Warehouse (Movement of air)						
Testtime	TRIO.BAS (to be qualified sampler)			MD8 AIR (qualified sampler method)		
Ora	Plate n.1	Plate n.2	Plate n.3	Plate n.1	Plate n.2	Plate n.3
cfu	cfu/m³	cfu/m³	cfu/m³	cfu/m³	cfu/m³	cfu/m³
10:35	20			30		
11:00		119			128	
11:27			94			87
	"A" cfu Average			"B" cfu Average		

Second test						
Environment: Warehouse (static air)						
Test time	TRIO.BAS (to be qualified sampler)			MD8 AIR (qualified sampler method)		
Hour	Plate n.1	Plate n.2	Plate n.3	Plate n.1	Plate n.2	Plate n.3
cfu	cfu/m³	cfu/m³	cfu/m³	cfu/m³	cfu/m³	cfu/m³
12:03	156			130		
12:29		116			91	
12:52			200			87
	"C" cfu Average			"D" cfu Average		

TRIO.BAS (To be qualified)		MD8 Air (Qualified)	
Average cfu A+C	117,5	Average cfu B+D	92,2

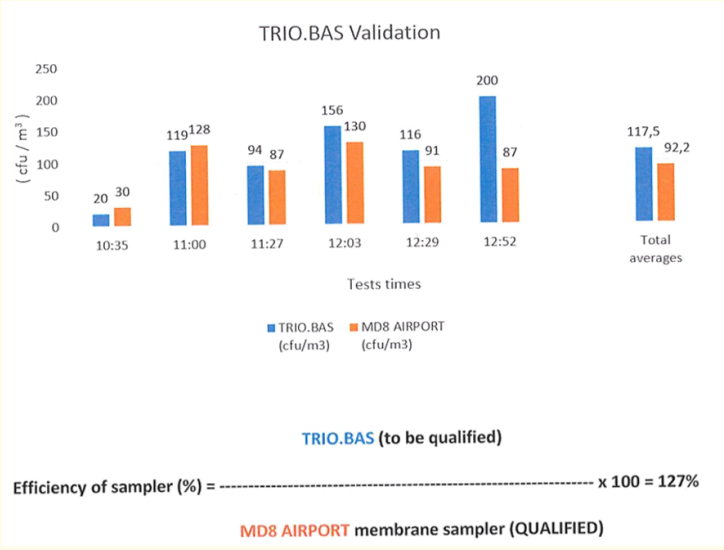

Figure 5

Conclusion

At the end of the tests carried out in the prepared structure, from the result s obtained, shown in the tables and from the calculation obtained with the application of the formula indicated in Chapter E.6.4 of the EN 17 141: 2020 standard, it was possible to highlight the following: The air sampler TRIO.BAS, as far as biological efficiency is concerned, while taking into account the losses caused both by the physical collection efficiency and by the effect that sampling has on the vitality of the microorganisms, has a good ability to collect particles carrying viable microorganisms: in fact, the found value of accept ability is included in the limit of acceptability of (100 +/- 50%) foreseen by the standard [1-4].

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