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APPLICATION NOTE
WHITE PAPER
EFFECT OF AGAR WEIGHT LOSS WITH CONTINUOUS ACTIVE AIR MONITORING ACCORDING GMP ANNEX 1 2023

ABSTRACT

The GMP Annex 1 2023 requests that microbial environmental monitoring by active microbial air samplers is actuated by continuous air monitoring. A prolonged sampling produces a loss of moisture in the agar of the Petri dish and therefore it is necessary that during the "continuous time" the dryness of the nutrient media is under control to guarantee a regular growing of the cfu.

The purpose of this paper is to evaluate if the agar (which volume was reduced with a treatment under sterile laminar flow before aspiration) has still the correct nutrient characteristics during the incubation of the Petri dishes.

The tested instrument was a AIR.BIO ONE air sampler with an aspiration rate of 25 litres / minute.

The results of the study are summarised in Table 1 and 2.

The average loss of humidity after 3 hours (=4.500 litres of air) in the first part of test under laminar flow was 29,7%.

In the second part of test, the Petri dishes aspirated the air in a normal open environment and the average growth after incubation (indicated in cfu/plate), in comparation with control plates with the original volume of agar, was 82,4%.

The acceptance criteria using the US Pharmacopeia Validation Recommendation for microbiological examination (chapter <1227>) is that the test plates had to recover < 70% of the challenge.

AIR.BIO ONE Microbial air sampler with an air flow rate of 25 l/m is therefore a suitable instrument for the microbial air monitoring when used in continuous monitoring.

KEY WORDS
Agar, Agar culture, cfu, Cultivability dehydration, E.M, Growth promotion test, TSA, UDAF, Unidirectional air flow

INTRODUCTION

The GMP Annex 1 2023 requests that microbial environmental monitoring by active microbial air samplers is actuated by continuous air monitoring. A prolonged sampling produces a loss of moisture in the agar of the Petri dish and therefore it is necessary that during the "continuous time" the dryness of the nutrient media is under control to guarantee a regular growing of the cfu.
The purpose of the present paper has to demonstrate which is the maximum loss of moisture (drying) to avoid an irregular growth of the cfu. Reduced access to moisture will reduce the growth-promoting properties of the culture medium leading to a failure of the plate to grow some or all of the microorganisms. This can lead to an underestimate of the number of microorganisms through loss of cultivability or viability.
Another important concern is with avoiding cracks in the agar which might render reading sections of the culture plate impossible.
Another point to be considered is the velocity of the air that impacts on agar surface.
The culture plates are typically 90 mm disposable Petri dishes filled with 26-30-34 ml of TSA.

TEST TO EVALUATE THE EFFECT OF WEIGHT LOSS IN 90 mm PETRI DISH

The principle of the test

-Material
AIR.BIO ONE Microbial air samplers - air flow 25 l/m
90 mm Petri dish with TSA agar medium – average weight 42,5 grams (30 ml).
Laminar flow bench

-The protocol of the test

The plates with 30 ml of TSA have a weight of 42,5 grams and were commercially available. After the first part of the test the plates were weighed to record the loss of moisture. A final weight was done at the end of incubation time.
All the plates (control plates and tested plates) were then used with active sampling to monitor the air of a normal environment (warehouse).
The result of the cfu count after incubation was then compared with the cfu count of the ALFA e BETA control plates to determine if the growth was acceptable.

-First part (Under a laminar flow bench with a speed of 0,45 m/sec) to evaluate the loss of water of each plate

The 90 mm Petri dish with the nutrient agar medium is exposed to a standard aspiration cycle of the air sampler under a sterile laminar flow for different times (e.g.: 1 hour = 1500 litres, 2 hours = 3000 litres, etc.). at ambient temperature (+21,0°C.; 1001 Hp; 40% Humidity).
The purpose is to evaluate and register the loss of humidity in aseptic conditions.

TABLE 1 - RESULTS OF LOSS WEIGHT (Dehydration)

TEST	PETRI DISH IDENTIFICATION	WEIGHT BEFORE THE TEST gr	WEIGHT AFTER 1 HOUR under laminar flow gr (1500 l)	WEIGHT AFTER 2 HOURS under laminar flow gr (3000 l)	WEIGHT AFTER 3 HOURS under laminar flow gr (4500 l)	WEIGHT AFTER INCUBATION (48 hours) gr	WEIGH LOSS AFTER 3 HOURS gr	% LOSS
Control plate	ALFA	42,70	-	-		42,20	0.5	-
Test 1	Plate “a”	42,60	38,60	34,60	30,6	30,1	12,5	29,3
Test 2	Plate “b”	42,70	38,70	34,70	30,7	30,2	12,5	29,3
Test 3	Plate “c”	42,60	38,60	34,60	30,6	30,3	12,3	28,8
Test 4	Plate “d”	42,60	38,60	34,60	30,6	30,1	12,5	29,3
Test 5	Plate “e”	42,70	38,70	34,70	30,7	30,2	12,5	28,1
Control plate	BETA	42,70				42,20	0,5	
Test 6	Plate “f”	42,60	38,60	34,60	30,60	30,10	12,5	29,3
Test 7	Plate “g”	42,60	38,80	34,50	30,20	29,70	12,9	3O,2
Test 8	Plate “h”	42,70	38,40	34,20	29,80	29,40	13,3	31,1
Test 9	Plate “i”	42,60	38,50	34,10	30,20	29,60	13,0	30,5
Test 10	Plate “l”	42,50	38,40	34,20	30,00	29,60	13,5	31,7

Average loss weight (Dehydration) = a, b, c, d, e = 28,9% - f, g,h, I, l = 30,5%
- 28,9+30,5=59,4 :2=
29,7%

First part of the test to reduce the moisture of
the nutrient agar

Second part of the test to evaluate the growth characteristics of the agar after dehydration

-Second part of the test to evaluate the growing performances related to the dehydration of the plates

The plates are then exposed to an aspiration cycle (e.g.: 1.000 litres of air) in a normal environment with natural environmental microorganisms (80-150 cfu /cubic meter) at ambient temperature (20°C), using the same air samplers.

This condition will demonstrate whether a plate retains the ability to support growth during the subsequent incubation time. The incubation time was 48 hours and the temperature 32°C.

Control plates (Control Plate “ALFA” and Control Plate “BETA”) that were not exposed to the laminar flow were used as a control.

TABLE 2 - RESULTS OF CFU GROWTH OF THE DEHYDRATED PLATES AFTER 4.500 LTS OF AIR

PLATE IDENTIFICATION	CFU CONTROL PLATE	CFU OF THE TESTED PLATE	CFU DIFFERENCE IN COMPARISON WITH CONTROL PLATE	GROWTH COMMENT %	% AVERAGE GROWTH
AIR SAMPLER ALFA					Plate "a", "b", "c", "d", "e" AVERAGE %
Plate "a"	Control Plate ALFA = cfu 105	88	88:105X100	83%	87,2%
Plate "b"		85	85:105X100	80%	
Plate "c"		76	76:105X100	72%	
Plate "d"		118	118:105X100	112%	
Plate "e"		94	94:105X100	89%	
AIR SAMPLER BETA					Plate "f", "g", "h", "i", "l" AVERAGE %
Plate "f"	Control Plate BETA = cfu 112	78	78:112x100	69%	77,6%
Plate "g"		105	105:112x100	93%	
Plate "h"		95	95:112x100	84%	
Plate "i"		75	122:112x100	66%	
Plate "l"		86	86:112x100	76%	

COMMENTS

Results of loss weight (Dehydration)

The average loss of weight is 29,7% after 4.500 lts.

Results of cfu growth

The acceptance criteria using the US Pharmacopeia Validation Recommendation for microbiological examination (chapter <1227>) is that the test plates had to recover < 70% of the challenge.

The average result is 87,2%+77,6% = 164,8 : 2 = 82,4%.

The result of 82,4% cfu growth, using TSA 90 mm culture Petri dishes with 30 ml of agar medium, justifies the average loss of 29,7% humidity and therefore the AIR.BIO ONE Microbial air sampler with an air flow rate of 25 l/m is the suitable instrument for the microbial air monitoring when used with continuous monitoring.

REFERENCE

1.EN 17141 Cleanrooms and associated controlled environments. Biocontamination control.
2. EU GMP Annex 1 Revision: Manufacture of Sterile Medicinal Products