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Effect of Agar Weight Loss with Passive Continuous Air Monitoring According GMP Annex 1 2023

Roberto Ligugnana*

President, Aroundlab, Via Novara 89, 20153 Milan, Italy.

***Correspondence:**
Roberto Ligugnana, President, Aroundlab, Via Novara 89, 20153 Milan, Italy, Tel: 00 39 02 48704496.

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ABSTRACT

The GMP Annex 1 2023 requests that microbial environmental monitoring by active and passive 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 of settle plate (which volume was reduced with a treatment under sterile laminar flow before aspiration) has still the correct nutrient characteristics during the incubation. The results of the study are summarised in Table 1 and 2.

The average loss of humidity after 4 hours in the first part of test under laminar flow was 14,7%. 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 87%. 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.

Keywords

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 and passive 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 point to be considered is how the air 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 with passive air monitoring

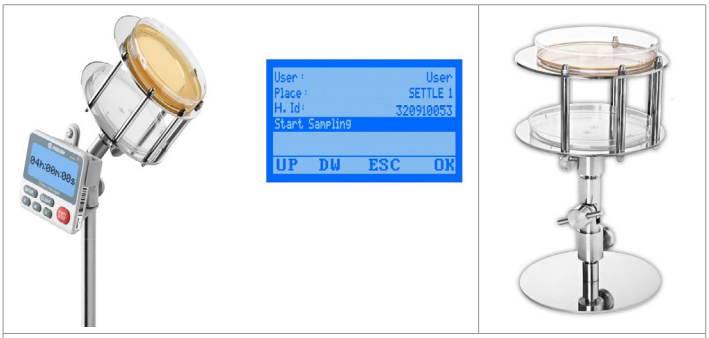
The principle of the test

-Material

90 mm Petri dish with TSA agar medium – average weight 42,5 grams (30 ml). TRIO SETTLE Support for Petri dishes

Laminar flow bench

Microbial air sampler 25 l/m air flow



Settle plate exposure under unidirectional air flow

The protocol of the test

The plates with 30 ml of TSA have an average 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 under a sterile laminar flow, for 4 hours at ambient temperature (+21,0°C.; 1001 Hp; 40% Humidity). The purpose is to evaluate and register the loss of humidity in aseptic conditions. The plates identified with the "a1", "b2", "c3", "d4", "e5" were positioned with the agar surface inclined under the laminar flow. The plates identified with the "f6", "g7", "h8", "i9", "l10" were positioned with the agar surface flat under the laminar flow.

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 TRIO.BAS air samplers. This condition will demonstrate whether a plate retains the ability to support a correct 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.

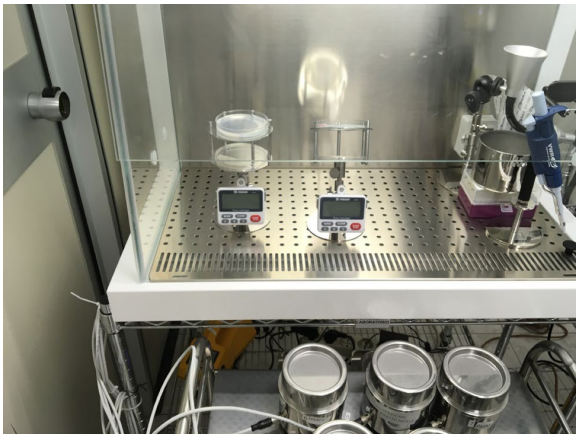
Comments

Results of loss weight (Dehydration) The average loss of weight is 14,7%. 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 [1,2].

Table 1: Results of Loss Weight (Dehydration).

TEST	PETRI DISH IDENTIFICATION	WEIGHT BEFORE THE TEST gr	WEIGHT AFTER 4 HOURS under laminar flow gr	WEIGHT AFTER INCUBATION (48 hours) gr	WEIGH LOSS gr	% LOSS
Control	Plate AA	42,40	-	41,90	0.50	-
Test 1	Plate "a1"	42,20	36,90	36,40	05,80	13,7
Test 2	Plate "b2"	42,20	36,70	36,20	06,00	14,2
Test 3	Plate "c3"	42,40	36,90	36,40	06,50	14,1
Test 4	Plate "d4"	42,40	36,80	36,20	06,20	14,6
Test 5	Plate "e5"	42,20	36,50	36,00	06,20	14,6
Control	Plate BB	42,40	-	41,90	0,5	-
Test 6	Plate "f6"	42,20	36,70	36,10	06,10	14,8
Test 7	Plate "g7"	42,40	36,90	36,40	06,00	14,1
Test 8	Plate "h8"	42,30	36,20	35,80	07,50	17,0
Test 9	Plate "i9"	42,20	36,60	36,00	06,10	14,6
Test 10	Plate "l10"	42,20	36,20	35,70	06,50	15,4

Average loss weight (Dehydration) = 14,24 % + 15,18% = 29,42:2 =14,7%



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

Table 2: Results of CFU Growth of the Dehydrated Plates after 3000 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 "a1"	Control Plate ALFA = cfu 140	125	125:140 X 100	89%	93,6%
Plate "b2"		115	115:140 X100	82%	
Plate "c3"		145	145:140 X100	103%	
Plate "d4"		155	155:140 X100	110%	
Plate "e5"		118	118:140 X100	84%	
Air Sampler Beta					Plate "f", "g", "h", "i", "l" AVERAGE %
Plate "f6"	Control Plate BETA = cfu 121	105	105:121 x100	86%	81%
Plate "g7"		95	95:121 x100	78%	
Plate "h8"		88	88:121 x100	72%	
Plate "i9"		110	110:121 x100	90%	
Plate "l10"		101	101:121 x100	83%	

The average result is 93,6% + 81,0% = 174,6 : 2 = 87%. The result of average 87 % cfu growth, using TSA 90 mm culture Petri dishes with 30 ml of agar medium, justifies the average loss of 14,7% humidity; the plates that were under the laminar flow in inclined position had better growing characteristics (93,6% vs 81,0%).

Reference

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