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Recovery of Microbial Contamination with Settle Plates Exposed in a Unidirectional Airflow Workstation for 4 Hours

T Eaton, K Capper, A Nash, S Drinkwater, J Bright - AstraZeneca, Macclesfield, UK

Corresponding Author: **Tim Eaton**, Sterile Manufacturing Specialist
AstraZeneca,
UK Operations,
Silk Road Business Park,
Macclesfield
Cheshire. SK10 2NA
England

Email: tim.eaton@astrazeneca.com
Telephone: +44(0) 1625 514916

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Summary

The ability of irradiated 90 mm diameter Tryptone Soya Agar (TSA) settle plates, exposed for 4 hours in a unidirectional airflow (UDAF) workstation (air supply velocity 0.45 m/s), to recover microbial contamination, was investigated. The investigation was completed by inoculation of numerous test plates with a range of different test micro-organisms and then exposure of the plates within a UDAF workstation for 4 hours. Following incubation, the numbers of recovered micro-organisms were compared with the numbers recovered from control plates that had been identically inoculated and incubated but not exposed within the workstation. Investigations of initial settle plate weight variation, loss of weight and water activity levels during exposure were also completed to help understand the influence of plate media water to any loss of microbial recovery. It was determined that the average number of recovered test plate colonies was reduced compared to the control plates and the exposure of the plates reduced the weight by an average of 10.85%. However, the media water activity levels remained consistently higher than the threshold level at which the growth of micro-organisms would be affected and the ability of the plates to recover micro-organisms was reduced but not significantly affected.

Key words: Microbiological settle plates, media dehydration, unidirectional airflow (UDAF), 4 hour exposure

1. INTRODUCTION

For the manufacture of sterile products, it is necessary to undertake microbiological monitoring of the cleanroom air, surfaces and personnel as part of the activities to determine that the environment is under adequate control. This is a requirement of the regulatory authorities and the limits to be applied to the monitoring methods are specified in the associated guidance documents [1,2]. The monitoring consists of some form of sampling and then an assessment of the number and identification of micro-organisms present in the sample There are a variety of sampling methods that can be used and the expectation is that these sampling methods should efficiently collect and recover micro-organisms and that the recovery efficiency is qualified. One method for the determination of the concentration of airborne microbial contamination, specified by the regulatory authorities, is the exposure of 90 mm diameter settle plates for a maximum period of 4 hours. There is however little available information to support this maximum exposure period and this method therefore needs evaluation for the conditions in which the plates are to be utilised. One of the main concerns relates to the desiccation of the plate microbial growth media, due to the flow of air, within the environment that is being sampled. A decrease in the media water content may result in a reduced recovery of any microbial contamination that is deposited onto it and exposure within a unidirectional airflow (UDAF) workstation, where there is a much higher flow of air over the plate compared with non-UDAF zones, would provide worst case challenge for loss of water.

Unpublished work previously completed at AstraZeneca Macclesfield assessed this issue by exposing numerous settle plates to UDAF for 4 hours and then inoculation of the plates with a range of test micro-organisms. Following incubation, the recovery was compared to that determined for identical control plates that had been similarly inoculated but had no exposure prior to incubation

and the results recorded a satisfactory recovery. However, it has been suggested that the addition of the test isolates to the media, as liquid suspensions, provides adequate conditions to rehydrate the growth media and ensure growth during incubation. Consequently, it was decided to utilise an alternative approach that negates the post exposure rehydration potential by firstly inoculating known concentrations of the test micro-organisms onto the media and then completing the 4 hour exposure period within a UDAF workstation. The recovered organisms were then compared to identically inoculated control plates that had been immediately lidded and had had no exposure within the workstation and were incubated simultaneously with the test plates following the 4 hour exposure. In this way, as both sets of plates were inoculated using the same liquid suspension method, the influence of only the airflow on the microbial recovery can be more readily assessed. Additionally, in order to further understand the effects of media desiccation on microbial growth, work that examined the weight variation of supplied plates, the level of plate water loss and the associated media water activity levels, following the exposure period, was also completed.

2. EXPERIMENTAL METHODS

A total of 3 trials (trials 1, 2 and 3) were completed. The same experimental methods, that are detailed below, were used for all 3 trials. The overall number of settle plates utilised for each trial was limited by the base area of the UDAF workstation utilised for the plate exposure.

Supplied Settle Plate Weight Variation

1. A total of 25 Becton Dickenson, BD BBL™ Soy Agar (TSA, Soybean-Casein Digest Agar Medium) 90 mm diameter plates (25g of medium, no surface neutralising agents), gamma irradiated and sealed in triplicate polythene bags, were weighed to determine the natural variation in weight, as supplied by the manufacturer. These plates were utilised for the test plates preparations (21 plates) and for the water loss investigations (4 plates) described in the following sections.

Test and Control Plates Preparations

2. Microbial test suspensions of known concentrations (target <100 cfu/ml) of a range of 7 different micro-organisms, that are detailed in the paragraph below, were utilised. Each test organism, from the same container, was inoculated onto 3 test plates and 2 control plates (a total of 21 test plates and 14 control plates for all 7 organisms, an overall total of 35 inoculated plates). Following inoculation, each of the 21 test plates were weighed.

3. The 21 test plates were de-lidded and exposed for 4 hours within a vertical unidirectional airflow (UDAF) workstation in a defined pattern covering the full workstation base area. Following the exposure, the test plates were immediately lidded, re-weighed and incubated at 32.5^0C ($\pm$ 1.5^0C) for 5 days.

4. The remaining 14 control plates were immediately lidded after inoculation and not subjected to exposure within the UDAF cabinet. These control plates were then placed in the same incubator, and at the same time, that was used to incubate the 21 test plates and simultaneously incubated under the same conditions.

5. At the end of the incubation period, all test plates were subject to enumeration and identification and for each micro-organism and for each trial, the average number of recovered colonies on the test plates, compared to the average number of colonies similarly recovered by the control plates.

Plates for Water Loss and Water Activity Investigations

6. A further 5 identical plates, that were not inoculated with test organisms, were utilised and 4 of these plates were weighed. For the fifth plate, the water activity (a_w) level was measured with a water activity meter and then all 5 plates were de-lidded and exposed within the UDAF workstation at the same time as the test plates. At the end of the 4 hour exposure, the 4 plates were re-weighed and the water activity level associated with the fifth plate was re-measured. The weight loss associated with the 4 plates was compared with the loss associated with 21 inoculated test plates to determine if the inoculation procedure had any influence on the plate water loss during the exposure period.

For trials 2 and 3, to address the possibility of any localised drying by the UDAF associated with the plate locations within the workstation, the exposed plates were re-arranged on the workstation base according to two pre-defined plate location patterns.

Test Micro-Organisms

A range of test micro-organisms, representative of a range of typical environmental bacteria and fungi, including two isolated from pharmaceutical production cleanrooms, were utilised for the microbial test suspensions. These organisms are detailed below.

Staphylococcus aureus	ATCC 6538	Gram-positive cocci bacteria, non-spore forming
Candida albicans	ATCC 10231	Yeast (fungi)
Pseudomonas aeruginosa	ATCC 9027	Gram-negative bacteria, non-spore forming
Bacillus subtilis	ATCC 6633	Gram-positive rod bacteria, spore forming
Aspergillus brasiliensis	ATCC 16404	Spore producing fungi
Staphylococcus epidermidis	Environmental isolate	Gram-positive cocci bacteria, non-spore forming
Aspergillis fumigatus	Environmental isolate	Spore producing fungi

Test Workstation

Vertical unidirectional airflow (UDAF) Class II microbiological safety cabinet. Air supply velocity, 0.45 m/s $\pm$ 20%, measured 15 cm from the terminal air supply HEPA filter. Cabinet air temperature 24^{o}C - 25.6^{o}C, relative humidity 47.9 % - 57.5%.

Test Results

The results associated with the supplied plate weight variations, plate weight losses, plate media water activity and test plate microbial recovery are shown in Annexes 1, 2 and 3 for trials 1, 2 and 3 respectively. Review of this data indicated it to be consistent throughout each of the 3 trials and summarised in tables 1, 2, 3, 4 and 5 is the information for each of the 3 trials relating to normal plate weight variation, inoculated plate weight loss, non-inoculated plate weight loss, plate media water activity, and test plate microbial recovery, respectively.

Table 1 As supplied plate weight variations (25 plates per trial, 75 plates in total)

Trial	Minimum plate weight (g)	Maximum plate weight (g)	Maximum plate weight variation* (%)	Standard Deviation
1	39.43	40.17	1.88	0.22
2	39.38	40.19	2.06	0.24
3	39.52	40.29	1.95	0.18
Overall average plate weight variation, trials 1, 2, 3			1.96	0.21

* The maximum plate weight variation is the worst-case difference in maximum and minimum weights divided by the minimum plate weight

Table 2 Average plate weight loss for plates inoculated with microbial test suspensions (21 plates per trial, 63 plates in total)

Trial	Average weight before exposure (g)	Average weight after exposure (g)	Average plate weight loss (%)
1	39.83	35.40	11.12
2	39.83	34.11	14.36
3	39.97	37.15	7.06
Overall average plate weight loss, trials 1, 2, 3			10.85

Table 3 Average plate weight loss for plates not inoculated with microbial test suspensions (4 plates per trial, 12 plates in total)

Trial	Average weight before exposure (g)	Average weight after exposure (g)	Average plate weight loss (%)
1	39.90	34.60	13.30
2	39.98	32.88	17.76
3	39.81	37.13	6.74
Overall average plate weight loss, trials 1, 2, 3			12.60

Table 4 Plate media water activity, a_w (1 plate per trial, 3 plates in total)

Trial	Water activity before exposure	Water activity after 4-hour exposure	Loss of water activity after 4-hour exposure (%)
1	0.9676	0.9663	0.13
2	0.9622	0.9654	-0.33*
3	0.9631	0.9575	0.58
Overall average plate loss of water activity, trials 1, 2, 3			0.13

*An increase in measured water activity following 4-hour exposure

Table 5 Average recovered microbial colonies (21 test plates per trial, a total of 63 test plates ; 14 control plates per trial, a total of 42 control plates)

Trial	Test micro-organism	Average recovered colonies test plate [a] (no.)	Average recovered colonies control plate [b] (no.)	Average recovery test plate vs. control plate (%)	Post incubation micro-organism identification (test plates)
1	*S. aureus*	8.0	15.0	53.3	*S. aureus*
	C. albicans	5.7	6.0	95.0	*C. albicans*
	P. aeruginosa	14.7	17.5	84.0	*P. aeruginosa*
	B. subtilis	8.0	11.0	72.7	*B. subtilis*
	A. brasiliensis	8.7	15.0	58.0	*A. brasiliensis*
	S. epidermidis *	223.3	237.0	94.2	*S. epidermidis*
	A. fumigatus *	1.3	2.0	65.0	*A. fumigatus*
	Average	38.5	43.4	88.7	-
2	*S. aureus*	10.7	22.5	47.6	*S. aureus*
	C. albicans	12.0	18.0	66.7	*C. albicans*
	P. aeruginosa	9.3	27.0	34.4	*P. aeruginosa*
	B. subtilis	4.7	8.5	55.3	*B. subtilis*
	A. brasiliensis	0.7	0.0	Indeterminate	*A. brasiliensis*
	S. epidermidis *	176.7	214.0	82.6	*S. epidermidis*
	A. fumigatus *	0.3	1.0	30.0	*A. fumigatus*
	Average	30.6	41.6	73.6	-
3	*S. aureus*	13.7	21.0	65.2	*S. aureus*
	C. albicans	16.0	17.5	91.4	*C. albicans*
	P. aeruginosa	20.0	25.0	80.0	*P. aeruginosa*
	B. subtilis	33.3	26.0	128.1	*B. subtilis*
	A. brasiliensis	14.3	19.5	73.3	*A. brasiliensis*
	S. epidermidis *	126.7	193.0	65.7	*S. epidermidis*
	A. fumigatus *	1.7	5.0	34.0	*A. fumigatus*
	Average	32.2	43.9	73.4	-
Averages Trials 1, 2, 3	*S. aureus*	10.8	19.5	55.4	-
	C. albicans	11.2	13.8	81.2	-
	P. aeruginosa	14.7	23.2	63.4	-
	B. subtilis	15.3	15.2	100.7	-
	A. brasiliensis	7.9	11.5	68.7	-
	S. epidermidis *	175.6	214.7	81.8	-
	A. fumigatus *	1.1	2.7	40.7	-
	Overall average	33.8	42.9	78.7	-

*Environmental isolates

a. 3 Test plates were utilised for each test micro-organism (21 plates per trial, a total of 63 plates in total).
b. 2 Control plates were utilised for each test micro-organism (14 plates per trial, a total of 42 plates in total).

2. DISCUSSION OF RESULTS

It can be seen from table 1 that the maximum plate weight variation for the 3 trials was 2.06% with an associated maximum standard deviation of 0.24, relating to trial 2. The weight variation also includes the variations associated with the plate and lid material as well as the media and is confirmed by table 1 to be very well controlled. When compared with the subsequent plate weight loss during the 4 hour exposure period, shown in table 2 and discussed later in this section, this weight variation is insignificant and therefore would not influence the extent of the recovery of any microbial contamination deposited onto the plates.

Table 2 shows the average reduction in plate weight, due to loss of water from the plate media over the 4 hour exposure period, associated with the test plates inoculated with the test micro-organisms. The overall average reported loss was 10.85% and the corresponding average weight loss associated with the plates that had not been inoculated, shown in table 3, was 12.60%. Additionally, a review of the individual inoculated test plate weights shown in tables A1.2 (Annex 1), A2.2 (Annex 2) and A3.2 (Annex 3) for trials 1, 2 and 3 respectively, confirms that 19 test plates had average weight losses that were greater than the average value recorded for the non-inoculated plates, with a maximum individual plate loss recorded to be 21.25% (test plate reference 2.1, table A2.2, Annex 2). It is therefore considered that the addition of the extra liquid, as part of the test inoculum suspension, had no significant effect in reducing the plate weight loss during the 4 hour exposure period and is therefore unlikely to influence the extent of recovery of any microbial contamination.

With regard to table 5, it can be seen that generally, the average number of recovered colonies is reduced for the test plates compared to the control plates. As the test inoculum added to both the test and control plates was from the same container and using good laboratory technique, it is reasonable to assume that any variation in the inoculation levels would not greatly influence this difference in the number of recovered colonies. The relationship of plate water loss with the actual recovery of the test micro-organisms can be determined by correlation of the average recovered numbers of colonies of the test micro-organisms on the test plates compared with the corresponding colonies on the control plates, that are shown in table 5. This information is included with table 6 which also reports the corresponding water activity values for each of the trials and shows that the highest average recovery (trial 1, 88.7%) was recorded with test plates that had a water loss of 11.12% which was not the lowest recorded water loss value. Conversely, the test plates that recorded the lowest water loss of 7.06% (trial 3) had only the marginally lowest average recovery (73.4%) compared with the recovery recorded for trial 2 (73.6%). For trial 2, associated with test plates that had the highest water loss (14.36%), the average test plate recovery that was not the lowest (73.6%). However, it is the water activity (a_w) level, and not the water content, that determines the ability to support microbial growth [3].

The lowest water activity level at which the majority of bacteria will grow is about 0.90. The water activity levels required for mold and yeast growth is about 0.61, and the lower limit for growth of mycotoxigenic molds is about 0.78 and with a water activity level below 0.6, microbial growth is not possible. It can be seen from table 4 that there is no significant change in the water activity levels following the 4 hour exposure period, with an overall average loss of 0.13% and a maximum loss of 0.58% (trial 3) which also recorded the lowest recorded water activity value of 0.9575. The water activity values all remained higher than the threshold level at which the growth of micro-organisms would be affected and are assessed to be more than satisfactory to support the growth of microbial contamination. Although it seems reasonable to assume that the reported levels of plate water loss may have had an influence on the extent of the recovery of any microbial contamination, the consistently high water activity levels maintained throughout may indicate that there could be other unknown factors to consider. The level of plate water loss required to reduce the water

activity level to a value that inhibits microbial growth is not known but from this program of work, water loss of up to 14.36 % does not influence the plate water activity levels.

Table 6 Recovery of test micro-organisms and corresponding plate weight and water activity losses

Trial	Average test plate recovery vs control plate after 4 hour exposure (%)	Average test plate weight loss after 4 hour exposure (%)	Average plate water activity loss after 4 hour exposure (%)
1	88.7	11.12	0.13
2	73.6	14.36	-0.33
3	73.4	7.06	0.58
Overall average	78.7	10.85	0.13

It should be noted, that for all 3 trials, both the test and control plates associated with *Staphylococcus epidermidis* had counts that exceeded the target inoculation level of < 100 cfu/ml per plate with an average highest count of 223.3 recorded in table 5 for trial 1. Overgrowth of environmental isolates is not unusual as they are prepared from a source plate, rather than a pre-prepared commercial suspension of defined concentration, and the plate preparation requires dilution which can lead to errors in attaining the required concentration. This is not considered to be an issue as the higher numbers could, with appropriate attention, still be counted with reasonable accuracy. The recovery of *A. fumigatus*, by both test and control plates, for all 3 trials was extremely low and very much lower than that associated with all of the other test micro-organisms. The reason for the extremely low numbers of colonies is unknown but could be associated with the type of standard non-selective TSA media that was utilised and a more selective media, specifically targeted for the growth of this micro-organism, may be more appropriate. With these extremely low colony numbers for both the test and control plates, meaningful information regarding the test plate recovery is not possible.

Shown in figure 1 are all of the actual (not the average values) test plate and control plate colony counts that are recorded in tables A1.5 (Annex 1), A2.5 (Annex 2) and A3.5 (Annex 3) for trials 1, 2 and 3 respectively. The recovered colony numbers are displayed, on a log scale, as 'panels 'where the red plotting symbols relate to the test plates and the control plates are indicated by the blue plotting symbols. This provides a readily informative illustration of the differences in recovered number of colonies and it can be seen that for *A. fumigatus*, although the recorded number of colonies are extremely low for both the test and control plates, the distributions are actually reasonably similar. Ignoring these low numbers for *A. fumigatus* which present issues for any statistical assessment, a 2-sample t-test analysis of the remaining data shows that the reduction in the number of recovered test plate micro-organisms compared to the control plate recovery is statistically significant ($p<0.05$) for just 2 of the micro-organisms, *S. aureus* and *P. aeruginosa*. This is confirmed by the overall average test plate recoveries for these 2 micro-organisms (55.4% and 63.4% respectively) that is shown in table 5. The reasons for these lower recoveries are not clear, particularly when reviewing the data associated with *S. epidermidis*, considered to have the most fastidious requirements for growth, but was recorded to have a superior average test plate recovery (81.8%) compared to both *S. aureus* and *P. aeruginosa.* However, these recorded recovery levels are consistent with the levels of variation typically seen with this type of microbiological data.

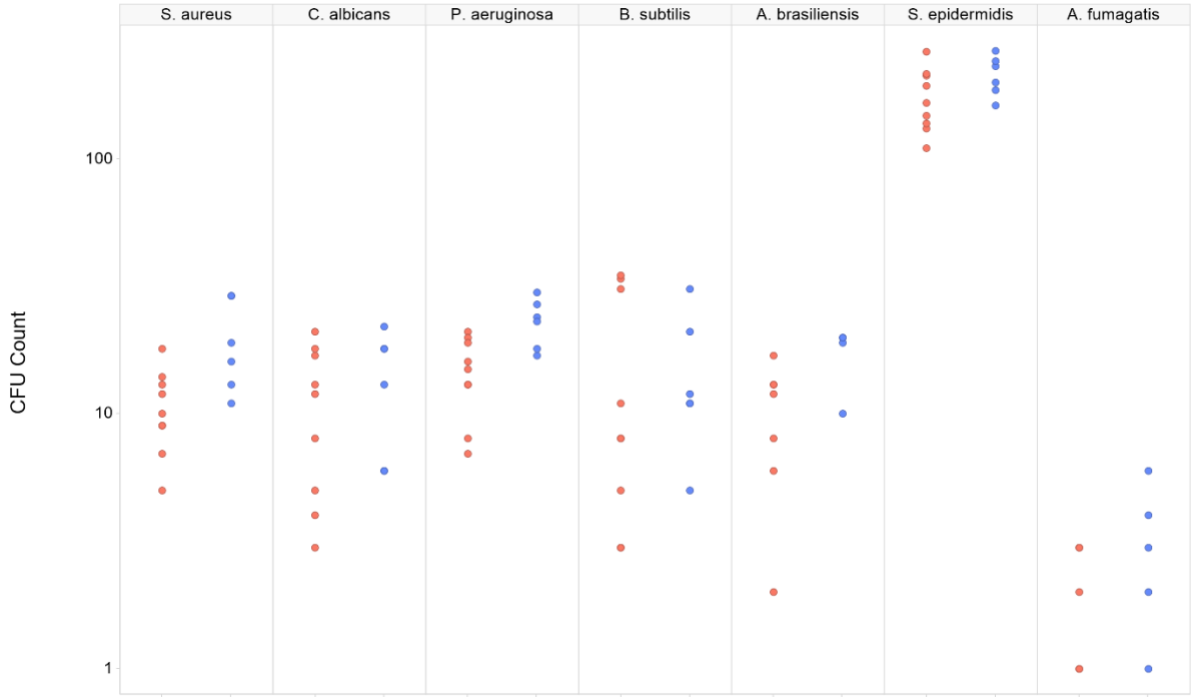

Figure 1 Recovered colonies of the test micro-organisms for test plates (red) and the corresponding control plates (blue)

In order to further assess the importance of the data and the implications for monitoring within a cleanroom environment utilised for sterile products manufacture, it is useful to understand the microbial contamination that is typically recovered from such a well designed and properly managed cleanroom. For several cleanrooms within 3 aseptic manufacturing facilities at AstraZeneca Macclesfield, the occurrences of *S. aureus* and Gram-negative organisms were reported to be very low, at 0.4% and 3.7% respectively, of the total recovered microbial population. For the test data associated with *S. aureus* and *P. aeruginosa*, highlighted to both have statistically significant reduced recoveries, the impact of these lower recoveries is therefore minimised. Fungal contamination is reported to be 1.1% and so, for the extremely low growth associated with *A. fumigatus* and the media used to recover it, the practical impact is therefore similarly also minimised. Gram-positive cocci are the predominant micro-organisms present within the cleanroom environments, with a reported occurrence of 73.0% and table 5 reports a high average recovery (81.8%) for *S. epidermidis*, which can also be clearly seen in figure 1. Consequently, the study shows that the exposure to 4 hours in a UDAF workstation (supply air velocity 0.45 m/s) does not significantly reduce the ability of the plates to recover micro-organisms. Exposure to UDAF presents worst case conditions for the plate media dehydration, compared with exposure within non-UDAF zones and such plates are therefore considered appropriate to use for monitoring of cleanrooms and workstations.

This experimental work is consistent with the unpublished work previously completed at AstraZeneca Macclesfield that assessed this issue by exposing numerous settle plates to UDAF for 4 hours and then inoculation of the plates with a range of test micro-organisms, before incubation and comparison with control plates. However, both of these methods predominantly utilise standard commercial test organisms and aqueous carrier to deposit the micro-organisms onto the media. A further evaluation could be completed if naturally occurring microbe-carrying particles were deposited directly onto the plates from an environment populated by personnel, that presents a high airborne microbial concentration. Following a short exposure in this type of environment, the plates can be exposed for 4 hours and compared to those similarly prepared plates that have no subsequent exposure. This type of study would provide further informative and complementary information on this topic.

3. CONCLUSIONS

Investigation of the loss of water from settle plates that had been separately inoculated with 7 different test micro-organisms and then exposed for 4 hours within a UDAF workstation reported variable media water weight losses with an average loss of 10.85%. The corresponding media water activity levels associated with this degree of water loss remained at levels at which the growth of micro-organisms should not be affected. Compared with the plates that had not been inoculated, the addition of test micro-organisms, as solution suspensions, to the settle plates did not significantly reduce the rate of water loss during the subsequent 4 hour exposure period that could have influenced the resultant recovery of the micro-organisms. A review of the corresponding numbers of inoculated test micro-organisms recovered by the test plates following the 4 hour exposure, and compared with the control plates, indicates an overall reduction. This is likely to be associated with the plate media water loss but there is no obvious direct correlation of microbial recovery as the highest recovery was recorded with plates that did not have the lowest loss of water. Although the water loss is likely to have an influence on the levels of recovery, the water activity levels are an indicator of the ability of the plates to recover microbial contamination and all levels remained consistently higher than the threshold value required for microbial growth. The amount of plate water loss needed to reduce the water activity level to a microbial growth inhibiting value is unknown. However, this program of work indicates that a loss of water of up to 14.36 % (by weight) does not influence the plate water activity levels but further work to investigate this relationship would be useful. The natural weight variations associated with the as supplied settle plates were found to be extremely low and insignificant compared to the weight loss during the 4 hour exposure and therefore considered not to influence the extent of the recovery of any microbial contamination deposited onto the plates.

With regard to the recovery of the test micro-organisms for the test plates compared to the control plates, for two of the test micro-organisms, *S. aureus* and *P. aeruginosa*, the reduction was confirmed to be statistically significant but the recorded recovery levels are considered to be within the usual levels of variation seen with this type of microbiological data. However, the occurrence of these types of micro-organisms in cleanrooms is typically less than 4%, with Gram-positive cocci (GPC) reported to be the predominant micro-organisms, with an occurrence of about 73.0%. Consequently, with the high average recovery reported for *S. epidermidis*, a typical readily occurring cleanroom GPC, the study shows that the exposure for 4 hours in a UDAF workstation, with an air supply velocity of 0.45 m/s, does not significantly affect the ability of the plates to recover micro-organisms. It is concluded that such plates are appropriate to use and would provide meaningful monitoring information.

4. RECOMMENDATIONS

The study is consistent with previous unreported work completed at AstraZeneca Macclesfield that inoculated commercial test micro-organisms onto plates using an aqueous carrier but after, and not before, a similar 4 hour exposure. However, both of these methods predominantly utilise standard commercial test organisms and aqueous carrier to deposit the micro-organisms onto the media. It is recommended that further work is undertaken with naturally occurring microbe-carrying particles typically dispersed from personnel and deposited directly onto test and control plates from the air without the need for a carrier medium. A portion of these plates should then be exposed in a similar UDAF workstation for 4 hours and the remaining plates used as controls to provide further useful information on this subject. It is also recommended that further work is undertaken to investigate the correlation between plate water loss and water activity.

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Annex 1

Trial 1 Results

Table A1.1 Supplied plate weight variations, SD 0.22 (25 plates)

Minimum plate weight (g)	Average plate weight (g)	Maximum plate weight (g)	Maximum plate weight variation* (%)
39.43	39.85	40.17	1.87

* The maximum plate weight variation is the worst case difference in maximum and minimum weights divided by the minimum plate weight

Table A1.2 Plate weight loss, plates inoculated with microbial test suspensions (21 test plates)

Test plate reference	Weight before exposure (g)	Weight after exposure (g)	Plate weight loss (g)	Plate weight loss (%)
1.1	39.93	33.49	6.44	16.13
1.2	40.01	36.11	3.90	9.75
1.3	40.10	36.64	3.46	8.63
1.4	40.17	35.49	4.68	11.65
1.5	39.97	36.29	3.68	9.21
1.6	40.07	34.36	5.71	14.25
1.7	39.66	36.53	3.13	7.89
1.8	39.56	34.9	4.66	11.78
1.9	39.57	33.96	5.61	14.18
1.10	39.56	35.72	3.84	9.71
1.11	39.61	36.44	3.17	8.00
1.12	39.97	36.57	3.40	8.51
1.13	40.08	34.13	5.95	14.85
1.14	39.99	35.92	4.07	10.18
1.15	39.97	36.60	3.37	8.43
1.16	40.15	35.38	4.77	11.88
1.17	39.75	33.04	6.71	16.88
1.18	39.71	35.39	4.32	10.88
1.19	39.62	36.1	3.52	8.88
1.20	39.65	35.04	4.61	11.63
1.21	39.43	35.31	4.12	10.45
Averages	39.83	35.40	4.43	11.12

Table A1.3 Plate weight loss, plates, not inoculated with microbial test suspensions (4 plates)

Weight before exposure (g)	Weight after exposure (g)	Plate weight loss (g)	Plate weight loss (%)
40.02	33.69	6.33	15.82
40.08	35.39	4.69	11.70
39.73	33.52	6.21	15.63
39.78	35.79	3.99	10.03
39.90	34.60	5.31	13.30
Averages			

Table A1.4 Media water activity, a_w (1 plate)

Water Activity before exposure	Water activity after exposure	Loss of water activity after 4 hour exposure (%)
0.9676	0.9663	0.13

Table A1.5 Average recovered microbial colonies (21 test plates, 14 control plates)

Test organism	Test plate reference	Recovered colonies test plate [a] (no.)	Recovered colonies control plate [b] (no.)	Post incubation micro-organism identification (test plates)
S. aureus	1.1	9	11	*S. aureus*
	1.2	5	19	*S. aureus*
	1.3	10	-	*S. aureus*
Average		8.0	15.0	Recovery 53.3%
C. albicans	1.4	8	6	*C. albicans*
	1.5	5	6	*C. albicans*
	1.6	4	-	*C. albicans*
Average		5.7	6.0	Recovery 95.0%
P. aeruginosa	1.7	16	17	*P. aeruginosa*
	1.8	15	18	*P. aeruginosa*
	1.9	13	-	*P. aeruginosa*
Average		14.7	17.5	Recovery 84.0%
B. subtilis	1.10	5	11	*B. subtilis*
	1.11	8	11	*B. subtilis*
	1.12	11	-	*B. subtilis*
Average		8.0	11.0	Recovery 72.7%
A. brasiliensis	1.13	6	10	*A. brasiliensis*
	1.14	12	20	*A. brasiliensis*
	1.15	8	-	*A. brasiliensis*
Average		8.7	15.0	Recovery 58.0%
S. epidermidis *	1.16	212	232	*S. epidermidis*
	1.17	264	242	*S. epidermidis*
	1.18	194	-	*S. epidermidis*
Average		223.3	237.0	Recovery 94.2%
*A. fumigatus**	1.19	1	3	*A. fumigatus*
	1.20	3	1	*A. fumigatus*
	1.21	0	-	*A. fumigatus*
Average		1.3	2.0	Recovery 65.0%

*Environmental isolates

a. 3 Test plates were utilised for each test micro-organism (a total of 21 plates for the full trial).
b. 2 Control plates were utilised for each test micro-organism (a total of 14 plates for the full trial).

Annex 2

Trial 2 Results

Table A2.1 Supplied plate weight variations, , SD 0.24 (25 plates)

Minimum plate weight (g)	Average plate weight (g)	Maximum plate weight (g)	Maximum plate weight variation* (%)
39.38	39.85	40.19	2.03

* The maximum plate weight variation is the worst case difference in maximum and minimum weights divided by the minimum plate weight

Table A2.2 Plate weight loss, plates inoculated with microbial test suspensions (21 test plates)

Test plate reference	Weight before exposure (g)	Weight after exposure (g)	Plate weight loss (g)	Plate weight loss (%)
2.1	39.67	31.24	8.43	21.25
2.2	39.65	35.78	3.87	9.76
2.3	39.71	35.79	3.92	9.87
2.4	40.02	33.22	6.8	16.99
2.5	40.16	32.22	7.94	19.77
2.6	40.10	36.09	4.01	10.00
2.7	39.89	35.38	4.51	11.31
2.8	39.65	34.14	5.51	13.90
2.9	39.92	33.89	6.03	15.11
2.10	40.05	36.04	4.01	10.01
2.11	40.15	34.16	5.99	14.92
2.12	40.19	33.22	6.97	17.34
2.13	39.56	32.44	7.12	18.00
2.14	39.70	35.73	3.97	10.00
2.15	39.67	35.49	4.18	10.54
2.16	40.13	32.83	7.30	18.19
2.17	39.89	31.88	8.01	20.08
2.18	39.66	35.69	3.97	10.01
2.19	39.43	35.89	3.54	8.98
2.20	39.38	33.40	5.98	15.19
2.21	39.79	31.80	7.99	20.08
Averages	39.83	34.11	5.72	14.36

Table A2.3 Plate weight loss, plates not inoculated with microbial test suspensions (4 plates)

Weight before exposure (g)	Weight after exposure (g)	Plate weight loss (g)	Plate weight loss (%)
40.08	33.62	6.46	16.12
40.05	33.46	6.59	16.45
39.94	32.07	7.87	19.70
39.83	32.36	7.47	18.75
39.98	32.88	7.10	17.76
Averages			

Table A2.4 Media water activity, a_w (1 plate)

Water Activity before exposure	Water activity after exposure	Loss of water activity after 4 hour exposure (%)
0.9622	0.9654	-0.33*

*****An increase in water activity

Table A2.5 Average recovered microbial colonies (21 test plates and 14 control plates)

Test organism	Test plate reference	Recovered colonies test plate [a] (no.)	Recovered colonies control plate [b] (no.)	Post incubation micro-organism identification (test plates)
S. aureus	2.1	7	29	*S. aureus*
	2.2	13	16	*S. aureus*
	2.3	12	-	*S. aureus*
Average		10.7	22.5	Recovery 47.6%
C. albicans	2.4	12	18	*C. albicans*
	2.5	3	18	*C. albicans*
	2.6	21	-	*C. albicans*
Average		12.0	18.0	Recovery 66.7%
P. aeruginosa	2.7	8	24	*P. aeruginosa*
	2.8	7	30	*P. aeruginosa*
	2.9	13	-	*P. aeruginosa*
Average		9.3	27.0	Recovery 34.4%
B. subtilis	2.10	3	5	*B. subtilis*
	2.11	8	12	*B. subtilis*
	2.12	3	-	*B. subtilis*
Average		4.7	8.5	Recovery 55.3%
A. brasiliensis	2.13	0	0	*A. brasiliensis*
	2.14	0	0	*A. brasiliensis*
	2.15	2	-	*A. brasiliensis*
Average		0.7	0.0	Indeterminate
S. epidermidis *	2.16	216	266	*S. epidermidis*
	2.17	166	162	*S. epidermidis*
	2.18	148	-	*S. epidermidis*
Average		176.7	214.0	Recovery 82.6%
A. fumigatus *	2.19	1	2	*A. fumigatus*
	2.20	0	0	*A. fumigatus*
	2.21	0	-	*A. fumigatus*
Average		0.3	1.0	Recovery 30.0%

*Environmental isolates

a. 3 Test plates were utilised for each test micro-organism (a total of 21 plates for the full trial).
b. 2 Control plates were utilised for each test micro-organism (a total of 14 plates for the full trial).

Annex 3

Trial 3 Results

Table A3.1 Supplied plate weight variations, SD 0.18 (25 plates)

Minimum plate weight (g)	Average plate weight (g)	Maximum plate weight (g)	Maximum plate weight variation* (%)
39.52	39.95	40.29	1.95

* The maximum plate weight variation is the worst case difference in maximum and minimum weights divided by the minimum plate weight

Table A3.2 Plate weight loss, plates inoculated with microbial test suspensions (21 test plates)

Test plate reference	Weight before exposure (g)	Weight after exposure (g)	Plate weight loss (g)	Plate weight loss (%)
3.1	39.91	37.77	2.14	5.36
3.2	39.93	37.72	2.21	5.53
3.3	40.07	36.41	3.66	9.13
3.4	40.02	37.91	2.11	5.27
3.5	39.86	35.86	4.00	10.04
3.6	39.79	34.23	5.56	13.97
3.7	39.94	37.64	2.30	5.76
3.8	40.01	38.81	1.20	3.00
3.9	40.21	38.62	1.59	3.95
3.10	40.09	37.74	2.35	5.86
3.11	40.05	38.61	1.44	3.60
3.12	39.84	37.43	2.41	6.05
3.13	39.86	36.92	2.94	7.38
3.14	40.29	38.16	2.13	5.29
3.15	40.13	37.98	2.15	5.36
3.16	40.02	36.64	3.38	8.45
3.17	39.66	36.42	3.24	8.17
3.18	39.77	35.87	3.90	9.81
3.19	39.92	38.69	1.23	3.08
3.20	39.98	36.51	3.47	8.68
3.21	40.1	34.28	5.82	14.51
Averages	39.97	37.15	2.82	7.06

Table A3.3 Plate weight loss, plates not inoculated with microbial test suspensions (4 plates)

Weight before exposure (g)	Weight after exposure (g)	Plate weight loss (g)	Plate weight loss (%)
40.12	40.08	0.04	0.10
39.52	36.63	2.89	7.31
39.70	38.38	1.32	3.32
39.89	33.42	6.47	16.22
39.81	37.13	2.68	6.74
Averages			

Table A3.4 Media water activity, a_w (1 plate)

Water Activity before exposure	Water activity after exposure	Loss of water activity after 4 hour exposure (%)
0.9631	0.9575	0.58

Table A3.5 Average recovered microbial colonies (21 test plates and 14 control plates)

Test organism	Test plate reference	Recovered colonies test plate [a] (no.)	Recovered colonies control plate [b] (no.)	Post incubation micro-organism identification (test plates)
S. aureus	3.1	9	13	*S. aureus*
	3.2	14	29	*S. aureus*
	3.3	18	-	*S. aureus*
Average		13.7	21.0	Recovery 65.2%
C. albicans	3.4	17	22	*C. albicans*
	3.5	18	13	*C. albicans*
	3.6	13	-	*C. albicans*
Average		16.0	17.5	Recovery 91.4%
P. aeruginosa	3.7	21	27	*P. aeruginosa*
	3.8	20	23	*P. aeruginosa*
	3.9	19	-	*P. aeruginosa*
Average		20.0	25.0	Recovery 80.0%
B. subtilis	3.10	34	31	*B. subtilis*
	3.11	35	21	*B. subtilis*
	3.12	31	-	*B. subtilis*
Average		33.3	26.0	Recovery 128.1%
A. brasiliensis	3.13	17	19	*A. brasiliensis*
	3.14	13	20	*A. brasiliensis*
	3.15	13	-	*A. brasiliensis*
Average		14.3	19.5	Recovery 73.3%
S. epidermidis *	3.16	132	186	*S. epidermidis*
	3.17	110	200	*S. epidermidis*
	3.18	138	-	*S. epidermidis*
Average		126.7	193.0	Recovery 65.7%
A. fumigatus *	3.19	3	6	*A. fumigatus*
	3.20	0	4	*A. fumigatus*
	3.21	2	-	*A. fumigatus*
Average		1.7	5.0	Recovery 34.0%

*Environmental isolates

a. 3 Test plates were utilised for each test micro-organism (a total of 21 plates for the full trial).
b. 2 Control plates were utilised for each test micro-organism (a total of 14 plates for the full trial).