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Peer Review Article: Recovery of Naturally Occurring Human Borne Microbial Contamination from Settle Plates Exposed in a Unidirectional Airflow Workstation for 4 Hours

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Recovery of Naturally Occurring Human Borne Microbial Contamination from Settle Plates Exposed in a Unidirectional Airflow Workstation for 4 Hours

Summary

The ability of irradiated 90 mm diameter tryptone soya agar settle plates, exposed for 4 hours in a unidirectional airflow (UDAF) workstation to recover microbial contamination, was investigated. The investigation was completed by direct contamination of numerous plates with naturally occurring microbe-carrying particles (MCPs) dispersed from a person within an enclosed environment. Half of these plates were subsequently exposed in a UDAF workstation for 4 hours and then incubated. The other half, contaminated with a similar number of MCPs, were not exposed in the workstation and provided the controls. Investigation of the reduction of plate weight during exposure was also completed to help understand the influence of plate media water content to any loss of microbial recovery. Following incubation, the numbers of recovered micro-organisms were compared and it was determined that the average number of recovered test plate colonies was reduced by 8.7% compared to the control plates and the exposure of the plates to UDAF for 4 hours reduced the plate weight, associated with loss of the media water, by an average of 12.3%. It was concluded that the ability of the plates to recover micro-organisms, following a 4 hour exposure to UDAF, was not significantly reduced.

Key words: Microbiological settle plate desiccation, media dehydration, unidirectional airflow (UDAF), 4 hour exposure, microbe-carrying particles (MCPs), environmental monitoring, settle plate sampling.

1. INTRODUCTION

For sterile products manufacturing, it is a requirement of the regulatory authorities that microbiological monitoring of the cleanroom air includes the use of 90 mm diameter settle plates exposed for a maximum validated period of 4 hours. The limits to be applied to this monitoring method are specified in the regulatory authority documents [1,2] and the expectation is that the sampling method should efficiently collect and recover micro-organisms and that supporting data for the recovery efficiency is available. A prime concern relates to the desiccation of the plate microbial growth media, due to the flow of air within the environment that is being sampled, that may reduce the recovery efficiency. However, apart from some information offered by the plate suppliers, there is little available data to support the stated maximum 4 hour exposure period. A decrease in the settle plate media water content may result in a reduced recovery of any microbial contamination that is deposited onto it. 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 environment for water loss and so reduce the likelihood of microbial growth.

Published work [3] assessed this issue by inoculation of plates with a range of test micro-organisms and then exposure to UDAF for 4 hours. Following incubation, the recovery was compared with that for identical plates that had been similarly inoculated but had no exposure to UDAF prior to incubation. Unpublished work, previously completed at AstraZeneca (Macclesfield), was similarly undertaken but with inoculation of the plates following the 4 hour exposure. Both of these methods recorded reduced but similar satisfactory levels of recovery of about 78%. However, each method utilised standard commercial test organisms and an aqueous carrier to deposit suspensions of micro-organisms onto the media and the carrier solution may have provided adequate conditions to rehydrate the media and ensure growth during incubation. Further work was recommended [3] using naturally occurring microbe-carrying particles (MCPs), dispersed from personnel, that are more representative of the majority of microbes recovered from the cleanroom, and deposited directly

onto test and control plates from the air without the need for a carrier medium. A method to provide plates contaminated with human borne MCPs, in sufficient and consistent numbers that could be used for such an evaluation, was derived. Subsequently, pre-work to confirm that the method could provide plates contaminated with sufficient MCPs is reported as the plate MCP verification study (Section 3). The main study, the comparison of contaminated plates exposed to UDAF (the test plates) with those that had no exposure (the control plates), is reported as the contaminated plate exposed in UDAF environment for 4 hours (Section 4). Additionally, in order to further understand the effects of media desiccation on microbial growth, the level of plate water loss, following the exposure, was also measured. Further work has also been undertaken to determine reductions in media water activity levels during similar exposure conditions and utilised to understand the influence that this parameter has on the plate microbial recovery efficiency.

2. METHOD TO CONTAMINATE SETTLE PLATES WITH MCPS

Fundamental to the successful evaluation of the recovery of human borne MCPs deposited onto the plates is the requirement to be able to provide an appropriate number of settle plates onto which have been deposited MCPs in sufficient numbers and in a short period of time to limit the dehydration of the media. The contaminated plates are to be subsequently divided into equal numbers of test and control plates, and so it is also a requirement that the two sets of plates collectively contain equivalent numbers of MCPs. However, initial attempts to provide such plates by exposure within busy office and laboratory environments returned plates with low and unacceptably variable numbers of MCPs.

In order to increase the numbers deposited, extended exposure periods were required with implications to media desiccation which, when subsequently utilised for exposure for the further 4 hours required for the testing, would not be representative of the actual initial media condition.

Consequently, an appropriate environment in which plates could be exposed, for a minimum amount of time, in order to collect sufficient and uniform numbers of MCPs (typically greater than 20 but less than 250) was further explored and a disused laboratory emergency shower chamber was identified as suitable. The chamber is approximately 2.5 m x 1 m x 1 m and has a front opening access door that can then be closed after the occupant has entered. Initial investigations were completed with an individual, wearing normal outdoor clothing, entering into the chamber and after closing the door, exposing settle plates located around the periphery of the chamber base. After exercising (walking on the spot at 1 step per second) for varying periods, the individual stopped moving and the plates were re-lidded, incubated and the numbers of recovered plate microbial colonies counted. It was determined that, without over exhausting the test individual, a reasonable number of colonies (>50) could be recovered, after exercising for 10 minutes and then resting for a further 1 minute prior to re-lidding the plates. The chamber, with the individual present and exercising, is shown in Figure 1. A total of 20 (1 to 20) plates were positioned around the periphery of the chamber, as shown in Figure 2.

Figure 1 Shower chamber with individual exercising **Figure 2** Plates (20) located on chamber base

3. PLATE MCP CONTAMINATION VERIFICATION STUDY

Experimental Procedure

In order to verify that plates could be contaminated with sufficient MCPs using the shower chamber, the following procedure was utilised and completed a total of 3 times (Trials 1, 2 and 3). All plates utilised were Becton Dickenson, BD BBL™ Soy Agar (TSA, Soybean-Casein Digest Agar Medium) 90 mm diameter (25g of medium, no surface neutralising agents), gamma irradiated and sealed in triplicate polythene bags. The plates are sourced from an approved supplier and are routinely tested for their ability to recover microbial contamination.

1. A total of 20 plates were removed from their wrappings, weighed and placed around the base of the periphery of the chamber floor. The positioning of the plates, on the chamber base, is shown previously in Figure 2.

2. Individual (male) entered the chamber, removed the lids from each plate wearing sterile latex gloves, and exercised for 10 minutes, rested for 1 additional minute, and then quickly re-lidded each plate. All plates were weighed following re-lidding.

3. All plates were simultaneously incubated, in the same validated incubator, at 30- 35^{0}C for 5 days and the number of microbial colonies counted and identification of the organisms from three randomly selected plates completed. Each plate was weighed following incubation.

4. A further single trial (Trial 4) was completed by exposing 20 plates, in the defined locations, for 11 minutes without the individual exercising in the chamber. All plates were incubated under the same conditions and identification of the organisms from three randomly selected plates also completed. Each plate was weighed following the shower chamber exposure and following incubation.

Test Results

Trials 1, 2 and 3
The average initial plate weights, post chamber exposure and post incubation average weights, and the recovered numbers of microbial colonies following contamination with human borne MCPs, for each trial, are shown in table 1. With the exception of a single yeast (fungal) colony recorded with Trial 3 (plate location 4), all colonies were bacterial. Contaminants from three randomly selected plates from each trial were subject to Matrix-assisted laser desorption/ionization-time of flight (MALDI-ToF) mass spectrometry identification and this confirmed the majority to be the expected Gram positive skin microbes. The identifications included;

Staphylococcus epidermidis, lugdunensis, cohnii, succinus
Micrococcus luteus
Microbacterium species
Brevibacterium luteolum, species
Kocuria species
Dietzia species
Bacillus licheniformis
Chryseobacterium species (Gram-negative)
Rhodotorula mucilaginosa (yeast, Trial 3, plate 4)

Table 1 Settle plate recovered microbial colonies following contamination with MCPs

Plate Location Reference	Recovered cfu (no.)		
	Trial 1	**Trial 2**	**Trial 3**
1	80	75	88
2	57	52	84
3	80	57	83
4	79	51	67*
5	79	45	97
6	60	60	82
7	43	68	99
8	81	42	94
9	82	62	110
10	73	52	138
11	116	59	95
12	52	79	93
13	57	64	95
14	64	40	90
15	58	64	116
16	44	56	101
17	52	65	89
18	60	51	84
19	45	48	94
20	42	52	95
Total	1304	1142	1894
Average cfu per plate	65	57	95
Average initial plate weight (g)	39.82	39.90	39.93
Post shower chamber exposure Average plate weight (g); loss (%)	39.56; 0.65	39.71; 0.48	39.75; 0.45
Post incubation (Relative to shower chamber exposure) Average plate weight (g); loss (%)	36.18; 9.14	36.32; 8.97	36.33; 9.02

*Includes a single fungal count

Trial 4

A total of 19 cfu (and a single mould) were recovered following exposure and incubation. There were average plate weight losses of 0.70% and 9.80% following the exposure within the shower chamber and after incubation, respectively. Contaminants from three randomly selected contaminated plates were subject to MALDI-ToF identification. The identifications included *Micrococcus luteus, Bipolaris* species, *Rhodotorula mucilaginosa* and *Neisseria subflava/perflava* and so some are likely to have been sourced from the tester as part of the plate lids removal and re-lidding activities in the shower chamber. As expected, these results confirm that the individual exercising in the shower chamber was the source of the majority of the MCPs deposited onto the plates and had no influence on the water reduction levels during the chamber exposure or subsequent incubation period.

Selection of Locations for Test and Control Plates

From the twenty plates distributed around the shower chamber for each trial, identification of ten locations to use as test plates and the remaining locations, with similar numbers of deposited MCPs, to be used as control plates, was undertaken utilising the data recorded in table 1. The test plates would subsequently be utilised for the 4 hour UDAF exposure and the control plates would have no such exposure. The recovered microbial plate counts for the three trials are shown in graphic form in figure 3 and it can be seen that the count distributions are random, with fairly large differences in the counts associated with the plates for each trial and also when the counts for the three trials are compared with each other. Consequently, it was assessed that it was most appropriate to simply compare plates located adjacent to each other as they would be most likely to have comparable numbers of deposited microbes, due to this similar positioning. Therefore, all of the ten odd location numbered plates (Set 1) would provide the test plates and the remaining ten even number plates (Set 2) used to provide the controls. The information relating to this approach, using the data recorded in table 1, is summarised in table 2. Consequently, this approach provides two sets of plates contaminated with MCPs in sufficient and comparable numbers that can be utilised for further experimental work to provide meaningful information regarding the effect of recovery for such plates exposed to UDAF.

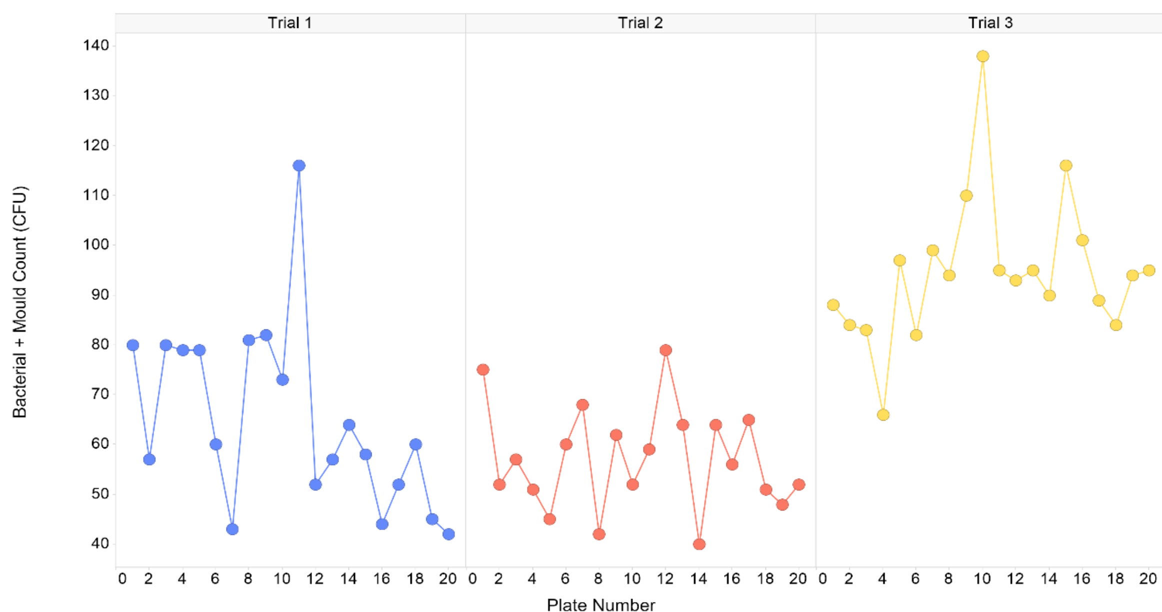

Figure 3 Recovered microbial plate counts distributions

Table 2 Settle plate recovered microbial colonies for allocated test (Set 1) and control plates (Set 2), following contamination with MCPs

Plate Location Reference		Recovered cfu (no.)					
		Trial 1		Trial 2		Trial 3	
Set 1 (Test)	**Set 2 (Control)**	**Set 1 (Test)**	**Set 2 (Control)**	**Set 1 (Test)**	**Set 2 (Control)**	**Set 1 (Test)**	**Set 2 (Control)**
1, 3, 5, 7, 9, 11, 13, 15, 17, 19	2, 4, 6, 8, 10, 12, 14, 16, 18, 20	692	612	607	535	966	928*
Average total count per 10 plates		652		571		947	
Total		1304		1142		1894	
Overall (Trials 1, 2, 3)		**Test Plates**			**Control Plates**		
Total		2265			2075		
Difference (Test – Control)		190					
Grand total (Test + Control)		4340					
% Difference (Difference/Grand total)		4.40					

* Includes a single mould count

4. CONTAMINATED PLATE EXPOSURE IN UDAF ENVIRONMENT FOR 4 HOURS

The plate MCP contamination procedure, outlined in the previous section, was again followed and was completed a total of 3 times (Trials A, B and C). On each occasion, all of the plates were immediately re-lidded and transferred to a vertical unidirectional airflow (UDAF) workstation, and laid on the base in a defined pattern as shown in figure 4. The workstation had an air supply velocity of 0.45 m/s (measured 6 inches from the filter face) and maintained average air temperature and relative humidity values of 21.9^{0}C and 41.8% respectively. The lids of the test plates (set 1) were removed and exposed for 4 hours, after which the test plates were immediately re-lidded, all plates were weighed and then incubated at 32-35^{0}C for 5 days. After incubation, all plates were weighed and the resultant number of microbial colonies counted.

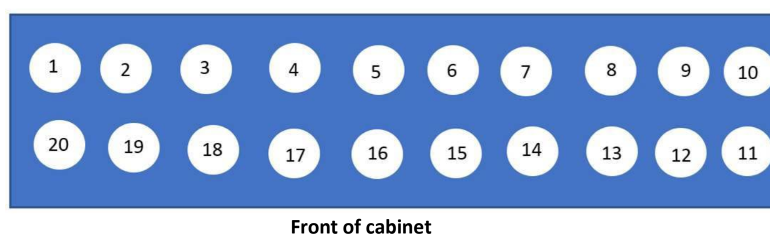

Figure 4 Distribution of settle plates on UDAF workstation during 4 hour exposure

Test Results

The recovered numbers of microbial colonies, following exposure and incubation of the test plates, and incubation only of the control plates, for each of the three trials, are shown in table 3. Also included are the average initial plate weights, post shower chamber and UDAF exposures and post incubation average weights. The recovered microbial plate counts and the plate weights are shown in graphic form in Appendix A in figures A1 and A2 respectively. These figures demonstrate the

random count distributions, and the varying plate weight losses associated with the exposure and incubation periods. For the recovered colonies, with the exception of a single yeast (fungal) colony recorded with three plates (Trial A location 5, Trial B location 17, Trial C location 13), all colonies were bacterial. Contaminants from three randomly selected plates from each trial were subject to identification (MALDI-ToF) and this confirmed the majority to be the expected Gram positive skin microbes. The identifications included;

Staphylococcus epidermidis, lugdunensis, cohnii, succinus
Micrococcus luteus
Microbacterium species
Brevibacterium luteolum, species
Kocuria species
Dietzia species
Bacillus licheniformis
Chryseobacterium species (Gram-negative)
Rhodotorula mucilaginosa (yeast, Trial C, plate 4)

Table 3 Settle plate recovered microbial colonies following contamination with MCPs

<table>
<tr><th colspan="2" rowspan="2">Plate Location Reference</th><th colspan="6">Recovered cfu (no.)</th></tr>
<tr><th colspan="2">Trial A</th><th colspan="2">Trial B</th><th colspan="2">Trial C</th></tr>
<tr><th>Test</th><th>Control</th><th>Test</th><th>Control</th><th>Test</th><th>Control</th><th>Test</th><th>Control</th></tr>
<tr><td>1</td><td>2</td><td>69</td><td>71</td><td>90</td><td>98</td><td>95</td><td>116</td></tr>
<tr><td>3</td><td>4</td><td>67</td><td>88</td><td>86</td><td>57</td><td>96</td><td>118</td></tr>
<tr><td>5</td><td>6</td><td>66*</td><td>49</td><td>85</td><td>81</td><td>88</td><td>109</td></tr>
<tr><td>7</td><td>8</td><td>46</td><td>96</td><td>95</td><td>88</td><td>107</td><td>101</td></tr>
<tr><td>9</td><td>10</td><td>81</td><td>93</td><td>95</td><td>109</td><td>90</td><td>121</td></tr>
<tr><td>11</td><td>12</td><td>48</td><td>57</td><td>149</td><td>82</td><td>89</td><td>123</td></tr>
<tr><td>13</td><td>14</td><td>74</td><td>67</td><td>131</td><td>83</td><td>72*</td><td>106</td></tr>
<tr><td>15</td><td>16</td><td>74</td><td>58</td><td>97</td><td>71</td><td>93</td><td>124</td></tr>
<tr><td>17</td><td>18</td><td>45</td><td>66</td><td>68*</td><td>75</td><td>74</td><td>93</td></tr>
<tr><td>19</td><td>20</td><td>38</td><td>65</td><td>58</td><td>82</td><td>67</td><td>118</td></tr>
<tr><td colspan="2">Average total count per plate</td><td>61</td><td>71</td><td>95</td><td>83</td><td>87</td><td>113</td></tr>
<tr><td colspan="2">Total</td><td>608</td><td>710</td><td>954</td><td>826</td><td>871</td><td>1129</td></tr>
<tr><th colspan="2">Overall (Trials A, B, C)</th><th colspan="3">Test Plates</th><th colspan="3">Control Plates</th></tr>
<tr><td colspan="2">Total</td><td colspan="3">2433</td><td colspan="3">2665</td></tr>
<tr><td colspan="2">Difference
(Control – Test)</td><td colspan="6">232</td></tr>
<tr><td colspan="2">% Reduction, Test vs. Control
(Difference/Control total)</td><td colspan="6">8.7</td></tr>
<tr><td colspan="2">Average initial plate weight (g)</td><td colspan="3">40.15</td><td colspan="3">40.07</td></tr>
<tr><td colspan="2">Post shower chamber exposure
Average plate weight (g); loss (%)</td><td colspan="3">39.92; 0.57</td><td colspan="3">39.82; 0.63</td></tr>
<tr><td colspan="2">Post UDAF exposure (Relative to shower chamber exposure)
Average plate weight (g); loss (%)</td><td colspan="3">35.02; 12.29</td><td colspan="3">39.71; 0.27</td></tr>
<tr><td colspan="2">Post incubation (Relative to UDAF exposure)
Average plate weight (g); loss (%)</td><td colspan="3">31.64; 9.62</td><td colspan="3">35.96; 9.45</td></tr>
</table>

*Includes a single mould count

5. DISCUSSION OF RESULTS

It can be seen from table 3 that for two of the individual trials (Trials A and C), and also when considering the three trials collectively, the average number of recovered colonies was reduced for the test plates compared to the control plates. However, the reduction was only 8.7% and when collectively considered, single analysis of the data (two-way Analysis of Variance) finds no significant difference between the control and test counts (p-value = 0.11). Published work [3] similarly assessed this issue but used standard commercial test organisms to inoculate plates, and not the naturally occurring MCPs, that are representative of the majority of microbes recovered from the cleanroom, reported for this work. An aqueous carrier was used to deposit suspensions of the commercial organisms onto the media and it was considered that the aqueous carrier may have provided conditions to rehydrate the media and ensure growth during incubation. With reduction in recoveries, relative to control plates, reported to be around 20%, it can be seen that when compared to the 8.7% reduction shown in table 3, this may not have been the case and the greater reduction may have been associated with the use of the aqueous carrier as a transfer medium or the use of commercial test organisms.

It can be seen with reference to table 3 that the overall average initial weights for the test and control plates were effectively the same (40.15g and 40.07g respectively) and similar water losses occurred after exposure within the shower chamber (0.57% and 0.63% respectively). Consequently, the subsequent overall average weight loss of plate water (12.29%) associated with the exposed test plates compared to the lidded control plates (0.27% reduction) following the 4 hours within the UDAF workstation can be assumed to be due to the drying action of the UDAF. The average plate weight losses following incubation were 9.62% and 9.45% for the test and control plates respectively and although the control plates had higher initial pre-incubation starting weights (plates remained lidded within the UDAF workstation), these reductions are effectively the same. It can be concluded that the reduced number of microbes recovered on the test plates could be associated with the increased water loss during the 4 hour exposure, or the actual exposure to the UDAF.

It is however the water activity (a_w) level, and not the water content, that determines the ability to support microbial growth [4]. The lowest water activity at which the majority of bacteria will grow is about 0.90. The a_w levels required for mould and yeast growth is about 0.61, and the lower limit for growth of mycotoxigenic molds is about 0.78 and with a a_w level below 0.6, microbial growth is not possible. Experimental work reported that, for the same type of settle plates and media used for this investigation, which had been similarly exposed for 4 hours under UDAF, there was no significant change in the a_w levels compared to plates that were not exposed. The average loss was 0.13% and the a_w values all remained higher (average 0.9643) than the threshold level at which the growth of micro-organisms would be affected.

Further work to investigate the distribution of a_w levels throughout the plate media, and the reduction in the levels was completed at AstraZeneca Macclesfield by exposing settle plates to UDAF. This work is summarised in Appendix B and involved sampling the plate media (agar) at several locations across the full surface area using a Biopunch® coring tool that enabled a small cylindrical sample to be taken of the full depth of the media. This sample was then cut in half to create two samples, one being representative of the surface media and the other representative of the base of the media. This confirmed no significant differences with the media water activities across the full surface of the plate and also between the surface and base samples i.e. the water activity levels were consistent throughout the entire volume of the media. Media a_w levels were determined for plates that had been exposed to UDAF for 2, 4, 6 hours and also for 336 hours (2 weeks) and compared with the a_w values measured for control plates where the lids remained in place during the exposure period. For the 2,4 and 6 hour plate exposures, the reductions in a_w levels were all extremely low with values recorded to be 0.01%, 0.32%, 0.51% respectively and all levels remained higher (lowest value 0.9584 for 6 hours exposure) than the threshold level (0.90) at which the growth of the majority of bacteria would be affected.

Graphical comparison of the a_w levels suggested a tentative negative correlation with the time the settle plate is exposed with a reduction rate of 1.07×10^{-3} of the initial a_w value (0.9713) per hour. The 336 hour plate exposure was completed to try and produce media with a significantly reduced a_w level and after this period of exposure, significant media shrinkage was observed with a measured average a_w value of 0.4448, a reduction of 54% compared to the control plate. If the determined reduction rate in the a_w value is assumed, the predicted a_w level after 336 hours of exposure would be 0.6118, which is an increase of 0.167 (37.5%) from the actual measured average value. Recognising the significant limitations of extrapolating beyond the 6 hour data point, this provides some crude indication of a_w level reduction and if this reduction rate is further applied, it can be determined that the theoretical time taken for the level to decrease to 0.90 is approximately 66 hours.

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 programme of work, water loss of up to 12.3%, as determined by the total plate weight reduction, does not influence the plate water activity levels. This is consistent with previously reported data that reported a similar water loss of 14.36 % that also did not similarly influence the a_w levels.

6. CONCLUSIONS AND RECOMMENDATIONS

Sterile tryptone soya agar settle plates inoculated with naturally occurring microbe-carrying particles (MCPs) dispersed from a person and exposed for 4 hours under UDAF (0.45 m/s) showed a 8.7% reduction in the number of recovered microbial colonies compared to similar plates that had no exposure to UDAF, and statistical analysis found this difference to be not significant. The corresponding plate weight reduction, associated with the loss of water from the media, was reduced by an average of 12.3% following the exposure of the plates to UDAF, following removal from the shower chamber. However, additional work investigating the media water activity levels confirmed no loss after such a 4 hour exposure and levels remained consistently higher than the threshold level at which the growth of micro-organisms would be affected. It was concluded that the ability of the plates to recover micro-organisms, following a 4 hour exposure to UDAF, was not significantly affected but the reported levels of plate water loss may have had an influence on the extent of this recovery. However, as the corresponding media water activity levels were maintained at very high levels throughout the 4 hour exposure period, it is possible that there could be other unknown factors to consider and it is recommended that further work is undertaken to investigate this.

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4. Scott, W.J. Water relations of food spoilage microorganisms. Adv Food Res 7:83-127. 1957.

APPENDIX A

CONTAMINATED PLATE COLONY AND WEIGHT DISTRIBUTIONS

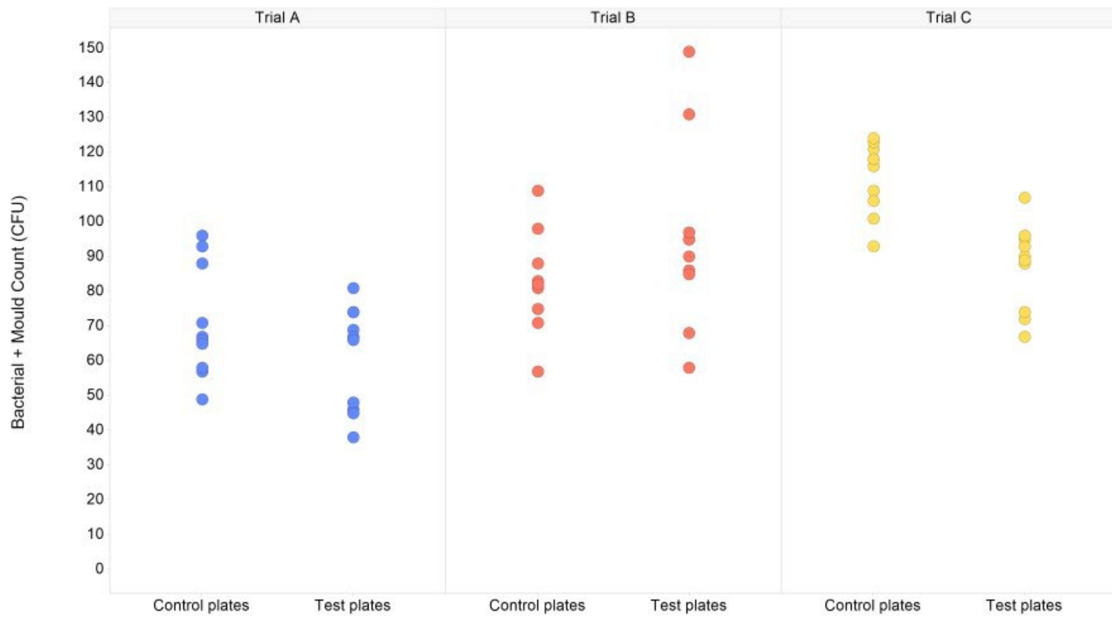

Figure A1 Contaminated plates colony distributions

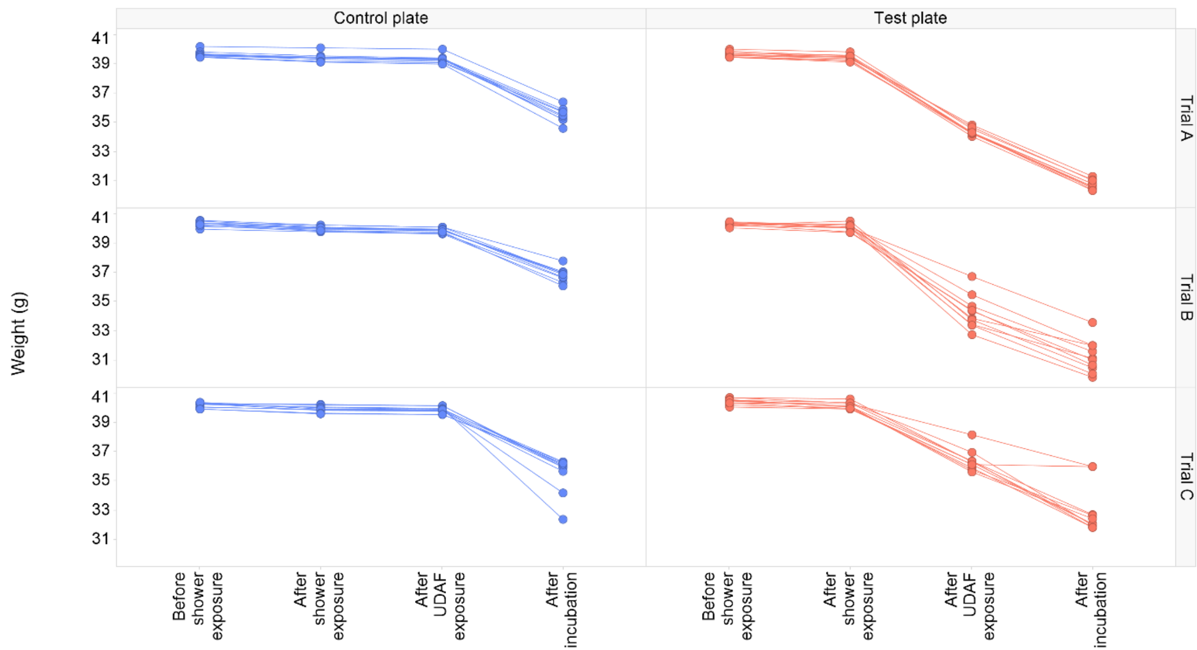

Figure A2 Contaminated plates weight distributions

APPENDIX B

INVESTIGATION OF PLATE MEDIA WATER ACTIVITY LEVELS

1. Distribution of water activity levels throughout plate media and 2 hour UDAF exposure

With reference to figure B1, five locations (1 to 5) of the settle plate media were sampled using a Biopunch® coring tool that enabled a small cylindrical sample to be taken of the full depth of the media. This was then cut in half using a scalpel to create two samples, one being representative of the surface media (sample a) and the other representative of the base of the media (sample b), to provide a total of ten samples (1a,b to 5a,b) for water activity (a_w) measurement. Three sample plates, Becton Dickenson, BD BBL™ Soy Agar 90 mm diameter plates (25g of medium, no surface neutralising agents), gamma radiated and sealed in triplicate polythene bags) were utilised. The plates were placed next to each other in a vertical UDAF workstation (air supply velocity 0.45 m/s), the lids removed and the media exposed for 2 hours. A fourth identical plate, but with the lid retained in place, was used as a control and simultaneously placed next to the other plates for the same period of time in the workstation. After exposure, the a_w levels were determined for each of the ten samples, from all of the four plates, using a water activity meter. The results are shown in table B1.

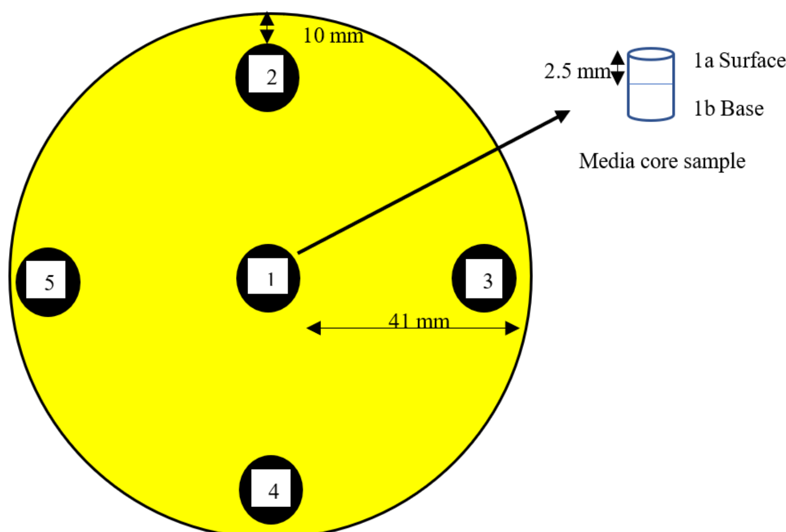

Figure B1 Plate media sampling locations

Table B1 Plate media water activity levels after 2 hour UDAF exposure

Media Sample Location	Water Activity (a_w)			
	Control	Plate 1	Plate 2	Plate 3
1a	0.9719	0.9711	0.9718	0.9736
1b	0.9713	0.9725	0.9739	0.9713
2a	0.9707	0.9711	0.9718	0.9723
2b	0.9699	0.9740	0.9735	0.9708
3a	0.9708	0.9710	0.9676	0.9712
3b	0.9706	0.9730	0.9679	0.9698
4a	0.9723	0.9705	0.9689	0.9706
4b	0.9720	0.9722	0.9725	0.9696
5a	0.9706	0.9697	0.9700	0.9701
5b	0.9706	0.9727	0.9664	0.9695
Averages	0.9711	0.9718	0.9704	0.9709
Average reduction vs. control (%)	-	-0.07	0.07	0.02
Overall average reduction vs. control (%)	-	0.01		

2. Investigation of 4 hour, 6 hour and 336 hour UDAF exposures

The experimental work described in the previous section was repeated separately for exposure times of 4 hours, 6 hours and 336 hours (2 weeks). The 2 week exposure period was included as the water activity reductions were found to be very low even after the 6 hour exposure and significant media shrinkage after 2 weeks was noted and therefore likely that the water activity levels would also be significantly affected. It should be noted that using the information recorded in the previous section, where it can be seen that there was negligible difference in the a_w levels between the different sample locations, a reduced but representative number of samples (locations 1 and 2) only were utilised. The results are shown in table B2.

Table B2 Plate media water activity levels after 4, 6 and 336 hour UDAF exposure

Media Sample Location	Water Activity (a_w)			
	Control	**Plate 1**	**Plate 2**	**Plate 3**
4 Hour exposure				
1a	0.9728	0.9689	0.9662	0.9668
1b	0.9678	0.9657	0.9676	0.9645
2a	0.9672	0.9667	0.9677	0.9680
2b	0.9717	0.9703	0.9649	0.9647
Averages	0.9699	0.9679	0.9666	0.9660
Average reduction vs. control (%)	-	0.21	0.34	0.40
Overall average reduction vs. control (%)	-	0.32		
6 Hour exposure				
1a	0.9732	0.9663	0.9695	0.9643
1b	0.9674	0.9646	0.9640	0.9584
2a	0.9714	0.9671	0.9664	0.9646
2b	0.9652	0.9637	0.9624	0.9613
Averages	0.9693	0.9654	0.9656	0.9622
Average reduction vs. control (%)	-	0.40	0.38	0.73
Overall average reduction vs. control (%)	-	0.51		
336 Hour exposure				
1a*	0.9662	0.4944	0.4432	0.4148
2a*	0.9688	0.4745	0.4275	0.4146
Averages	0.9675	0.4845	0.4354	0.4147
Average reduction vs. control (%)	-	49.92	55.00	57.14
Overall average reduction vs. control (%)	-	54.02		

*Due to the reduced thickness of the media, surface samples only could be obtained

3. Discussion of Results

The work completed for the 2 hour plate exposure confirmed no significant differences with the media a_w levels across the full surface of the plate and also between the surface and underside samples i.e. the a_w levels were consistent throughout the entire volume of the media with a maximum variation recorded for plate 2 (locations 1b and 5b) of 7.5×10^{-3}, which is 0.77% of the a_w activity value for this plate. The overall average reduction in the a_w level of the exposed plates compared with the control plate is of 0.01% and this is considered to be negligible. For the 4 hours and 6 hours exposures, the a_w levels were also reasonably consistent throughout the media with a maximum variation recorded for the 6 hour exposure with plate 2 (locations 1a and 2b) of 7.1×10^{-3}, which is 1.87% of the average a_w value for this plate. The overall average reductions in the a_w levels of the exposed plates compared with the control plates were 0.32% and 0.51% for the 4 and 6 hour exposures respectively and these are very low levels of reduction. For the 2 weeks (336 hours) exposure period, significant media shrinkage was noted and the a_w levels were measured, but as the depth of media was reduced, only the surface could be sampled. The average value of the six

measurements was determined to be 0.4448, a reduction of 54% compared to the average value of 0.9675 for the two control plates.
The average media a_w values for each of the three plates, for the 2, 4 and 6 hour plate exposures recorded in tables B1 and B2 are shown in graphical format in figure B2. This suggests a tentative negative correlation between the time the settle plate is exposed and the a_w level of the media and the best fit line graph indicates a decay rate of 1.07×10^{-3} per hour with an initial a_w level of 0.9713. If these numbers are utilised, the predicted a_w level after 336 hours of exposure would be 0.6118, an increase of 0.167 (37.5%) from the actual measured average value of 0.4448. Although it seems reasonable to assume a near linear reduction of plate media a_w with exposure to UDAF over a 6 hour period, for extrapolated times beyond this period, the correlation therefore appears to be much less evident, although likely to provide some reasonably meaningful information.
The lowest a_w value at which the majority of bacteria will grow is about 0.90. The a_w 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 a_w level below 0.6, microbial growth is not possible. For the 2,4 and 6 hour plate exposure times, the average a_w all remained higher (lowest 0.9622, plate 3 location 1b, at 6 hours) than the threshold level at which the growth of micro-organisms would be affected and suggests microbial recovery after a 6 hour exposure to UDAF would not be compromised. Assuming the constant decay rate of 1.07×10^{-3} of the a_w level per hour, the theoretical time taken for the a_w level to decrease to 0.90 is between 66 and 67 hours.

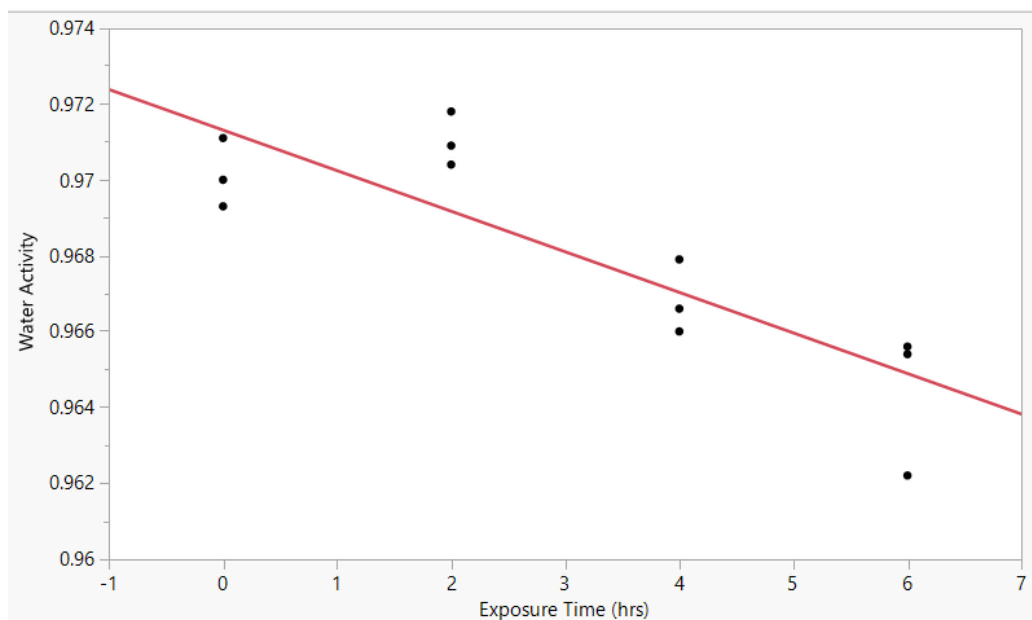

Figure B2 Plate media water activity levels after 2, 4 and 6 hours UDAF exposure