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AN INVESTIGATION OF THE ANTIMICROBIAL EFFICIENCY OF
MATRIX STEAM AND VACUUM CLEANING SYSTEMS

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1.0 Introduction

Matrix cleaning systems Ltd are in the business of providing technological solutions for the sanitisation of industrial and medical environments. This report details the results and findings of laboratory trials relating to the performance of the Matrix 8 bar and 4 bar steam and vacuum cleaning systems. All of these systems are intended for cleaning flat surfaces.

These devices consist of a mobile steam generation unit and waste collection unit to which a tube assembly is connected through which steam is delivered to a work head and by which a vacuum suction system removes treated debris from the working environment. Debris is generated during use and is collected in a waste vessel, which is intended to be maintained in a sanitary state by the addition of a disinfectant agent.



The objectives of the work reported here were as follows:

- A) To quantify the antimicrobial performance of each device against a range of medically significant organisms with respect to the cleaning of soiled surfaces.
- B) To assess the performance of a range of candidate antimicrobial substances with regard to the sanitisation of the debris collection vessel.
- C) To determine the existence and quantify the nature of any undesirable cross contamination events during operation.
- D) To describe and provide measurement of the temperature dynamics inside and below the work head treatment area during usage

2.0 Conditions; Flat Surface Cleaning Trials

Assessment of Test Surfaces and Debris reservoir

The test surface consisted of a 2-m² sheet of stainless steel plate (6 mm thick) onto which 48 X 25 cm² numbered quadrants had been etched. This etched matrix established the sampling template for recovery of organisms.

Contamination of the test surface was achieved by the application of an organic matrix containing suitable numbers microorganisms or spores. The organic matrix was manufactured with ¼ strength Ringers or Thigycollate solution (in the case of Clostridia) containing 3 % Albumin (Cohn fraction) and 2 % Lecithin.

After addition of challenge culture the matrix was mixed and applied by roller to the test surface after which the system was allowed to dry for 24 hours at ambient temperature. In this manner a dry film of microbiologically contaminated debris was obtained on the test surface. All challenge organisms were applied in monoculture or as groups of types within the same genus.

At the end of a cyclic cleaning cycle sequentially involving all test organisms 150 ml of the process liquor was recovered from the waste chamber. After creating a 1/10 dilution (in Universal quenching agent) and creating appropriate dilution series, the analysis of residual contamination was determined as described above.

3.0 Recovery of organisms

Attempts to recover organisms from the test surface were conducted prior to and after treatments involving steam cleaning.

For each sampling effort 6 X 25 cm² quadrants were designated by random number. Each designated area was swabbed with a transport swab pre-moistened in either ¼ strength ringers or Thioglycollate medium (Clostridial recovery).

Charged swabs were vortexed (1 minute) in either ¼ strength ringers or Thioglycollate medium prior to the creation of a decimal dilution series. The recovery effort proceeded by spiral plating (50 ul log) of the each member of the dilution series on an appropriate agar for the challenge organism. Additionally recovery from the 1 /10 test dilution of treated surfaces was achieved employing membrane filtration followed by whole membrane incubation on the appropriate agar and a non selective medium. Standard incubation conditions applied. Confirmation of isolates was achieved by biochemical typing, serology and where appropriate by genomic analysis.

In the case of the Debris reservoir liquor, after creating a 1/10 dilution (in Universal quenching agent) and creating appropriate dilution series, the analysis of residual contamination was determined as described above.

4.0 Measurement of operational characteristics

Temperature measurements were conducted on both the test surface and in the steam delivery environment of the cleaning head.

In the case of surface measurements thermistors were arranged through holes cut in a section of test surface 1 meter long and 0.75 meter wide. These were arranged perpendicular to the line of motion to of the cleaning head at 50 cm intervals Thermal measurements were taking during reciprocating motion of the cleaning head over the test surface for a period of 1 minute.

In the case of the cleaning head, a thermistor was located at the geometric centre of the device and a further two thermistors were located at points where steam vented from the delivery tube.

Data was recovered by data logger at a sampling rate of 3 measurements per second.

In this manner we able to measure the exit temperature of steam from the delivery tube and that of the internal environment of the cleaning head.

Measurement of the thermal dynamics of steam treated surfaces was conducted as a separate trial from the challenge work and the performance with and without vacuum on is reported

5.0 Form of Use of the Matrix Steam Cleaning devices

In all trials the machine under evaluation was charged with tap water. The device was powered up and allowed to stabilise until the steam ready indicator actuated. All trials surface challenge trials were commenced with an empty, sanitised debris bucket charged with either 100 mls of 10 % solution of Aqualin ECO F or dry but containing 4 X 20 gram Sodium dichloroisocyanurate tablets.

Trials were conducted on challenged surfaces both with vacuum in operation.

For any one test organism, a total of four contaminated surfaces were examined. Each surface was cleansed with the steam device according to the manufacturers instructions and each replicate was performed by the same operator. In practise the surface was treated until it was visually clean which on average accounted 60 seconds treatment under these conditions.

6.0 Test organisms

All organisms employed in this work were obtained as clinical or industrial isolates. During the trial working cultures were maintained on non selective agar slopes under appropriate conditions of storage.

Spore stocks of the Clostridium difficle strains were generated by recovery of the sediment from exponential liquid cultures which had been shocked with 96 % Ethanol for 50 minutes

Microbial suspensions intended for challenge work were prepared by recovery of cell mass from mid exponential liquid cultures after centrifugation. Cells pellets were washed and re-suspended in Ringers solution prior to adjustment to the desired cell density by Nephelometry.

Organism	Primary recovery medium
Clostridium difficle (Mixed vegetative/spore A B 02)	cycloserine-cefoxitin fructose agar
Clostridium difficle (Spore Form 3 strains A B 02)	cycloserine-cefoxitin fructose agar
Acinetobacter lwoffii	Leeds Acinetobacter Medium
Mycobacterium gordonae	Blood agar
Aspergillus niger	Rose Bengal Chloramphenicol agar
S.aureus (MRSA 3 strains)	Oxoid Chromogenic agar
Ecoli verotoxic 0157:H7	Sorbitol Mackoney agar
Group A Streptococci (mixed species)	Columbia agar
Bacillus cereus	PEMBA
Listeria monocytogenes	ALOA Agar & Palcam Agar
Salmonella seftenberg	XLD agar
Candida albicans	Rose Bengal Chloramphenicol agar
Pseudomoas aeruginosa	C-N agar

7.0 Environmental monitoring

During the treatment of contaminated surfaces air monitoring was conducted on agar surfaces employing an impaction device at a sample rate of 100 litres/atmosphere per minute. Additionally open agar plates were radiated around the axes treatment area commencing at 0.1 metres from the periphery of the treatment area and then at intervals 0.25, 0.5, 0.75 and 1 metre. These test were performed to detect any environmental cross contamination due to steam-generated aerosols during steam/vacuum operation.

8.0 Results

Table 1

Mean thermal performance in the deliver head data of the 8 Bar device and 4 Bar device during standard use.

DEVICE	Mean Steam Vent 1 'C	Mean Steam Vent 2 'C	Centre 'C	Vacuum
8 Bar	99.7	99.4	97.2	On
4 Bar	99.4	99.3	96.2	On
8 Bar	99.6	99.3	98.7	Off
4 Bar	99.5	99.6	98.2	Off

Table 2

Mean thermal performance of a stainless track during standard use of the 8 Bar and 4 Bar device during standard use with and without vacuum on.

Vacuum Off	8 Bar	4 Bar
Pre start up	22.6 'C	22.4 'C
Maximum (1 Pass)	81.9 'C	81.1 'C
Treated area after 5 seconds after head had passed	68.3 'C	66.4 'C
Single Point time above 80 'C during 1 minute treatment of the test piece	7.3 seconds	7.0 seconds
Single Point time above 72 'C during 1 minute treatment of the test piece	12.8 seconds	11.2 seconds
Maximum (1 Pass) intensive	98.2	97.6

Vacuum On	8 Bar	4 Bar
Pre start up	23.4 'C	22.9 'C
Maximum (1 Pass)	80.6 'C	80.1 'C
Treated area after 5 seconds after head had passed	67.5 'C	62.4 'C
Single Point time above 80 'C during 1 minute treatment of the test piece	6.6 seconds	6.3 seconds
Single Point time above 72 'C during 1 minute treatment of the test piece	11.3 seconds	10.9 seconds
Maximum (1 Pass) intensive	97.4	96.2

Table 3

Performance of the 8 Bar in the decontamination of challenged stainless steel surfaces by the application of steam and vacuum processing

Organism	Mean Challenge level cm ²	Mean Survivor level cm ²	Log Removal	% Removal
Clostridium difficile (Mixed vegetative/spore A B 02)	2.3E+08	0	8.7	>99.999
Clostridium difficile (Spore Form 3 strains A B 02)	4.1E+08	2	8.2	>99.999
Acinetobacter Iwoffii	3.2E+08	2	8.3	>99.999
Mycobacterium gordonae	2.7E+08	1	8.3	>99.999
Aspergillus niger	2.8E+08	1	8.7	>99.999
S.aureus (MRSA)	4.7E+08	1	8.8	>99.999
Ecoli verotoxic 0157:H7	3.6E+08	1	8.8	>99.999
Group A Streptococci (mixed species)	4.8E+08	1	8.5	>99.999
Bacillus cereus	1.1E+08	0	8.9	>99.999
Listeria monocytogenes	4.7E+08	2	8.4	>99.999
Salmonella seftenberg	1.6E+08	1	8.4	>99.999
Candida albicans	1.7E+08	1	8.2	>99.999
Pseudomonas aeruginosa	3.0E+08	2	8.3	>99.999

Table 4

Performance of the 4 Bar in the decontamination of challenged stainless steel surfaces by the application of steam and vacuum processing

Organism	Mean Challenge level cm ²	Mean Survivor level cm ²	Log Removal	% Removal
Clostridium difficile (Mixed vegetative/spore A B 02)	3.8E+08	2	8.3	>99.999
Clostridium difficile (Spore Form 3 strains A B 02)	2.6E+08	3	8.0	>99.999
Acinetobacter Iwoffii	3.9E+08	2	8.4	>99.999
Mycobacterium gordonae	1.8E+08	1	8.3	>99.999
Aspergillus niger	2.2E+08	1	8.3	>99.999
S.aureus (MRSA)	2.5E+08	0	8.9	>99.999
Ecoli verotoxic 0157:H7	4.6E+08	2	8.3	>99.999
Group A Streptococci (mixed species)	2.7E+08	1	8.3	>99.999
Bacillus cereus	2.2E+08	1	8.4	>99.999
Listeria monocytogenes	2.0E+08	0	8.6	>99.999
Salmonella seftenberg	2.3E+08	1	8.4	>99.999
Candida albicans	1.7E+08	1	8.4	>99.999
Pseudomonas aeruginosa	1.8E+08	0	9.1	>99.999

Table 5

Microbiological status of the contents of the debris reservoir after 13 sequential cleaning cycles of the test environment involving each test culture

Organism	No Biocide	Aqualin Dosed	Sodium dichloroisocyanurate
	Mean recovery cfu/ml	Mean recovery cfu/ml	Mean recovery cfu/ml
<i>Clostridium difficile</i>	1.30E+04	<10	<10
<i>Acinetobacter lwoffii</i>	8.20E+05	<10	<10
<i>Mycobacterium gordonae</i>	2.10E+04	<10	<10
<i>Aspergillus niger</i>	7.30E+04	<10	<10
<i>S.aureus</i> (MRSA)	2.30E+04	<10	<10
<i>Ecoli</i> verotoxic 0157:H7	90	<10	<10
Group A Streptococci (mixed species)	1.30E+05	<10	<10
<i>Bacillus cereus</i>	8.90E+04	<10	<10
<i>Listeria monocytogenes</i>	70	<10	<10
<i>Salmonella seftenberg</i>	45	<10	<10
<i>Candida albicans</i>	9.30E+05	<10	<10
<i>Pseudomonas aeruginosa</i>	6.10E+05	<10	<10

Table 6

Microbiological status of the peritreatment environment during treatments with the 8 Bar and 4 Bar devices.

8 Bar Vacuum On	Air Sampling	Plate 0.1 m	Plate 0.25 m	Plate 0.5 m	Plate 0.75 m	Plate 1.0 m
Organism	cfu/ 100litres	cfu/plate	cfu/plate	cfu/plate	cfu/plate	cfu/plate
<i>Clostridium Difficile</i>	<1	<1	<1	<1	<1	<1
<i>Acinetobacter lwoffii</i>	<1	<1	<1	<1	<1	<1
<i>Mycobacterium gordonae</i>	<1	<1	<1	<1	<1	<1
<i>Aspergillus niger</i>	17	<1	<1	<1	<1	<1
<i>S. aureus</i> (MRSA)	<1	<1	<1	<1	<1	<1
<i>Ecoli</i> verotoxic 0157:H7	<1	<1	<1	<1	<1	<1
Group A Streptococci (mix)	<1	<1	<1	<1	<1	<1
<i>Bacillus cereus</i>	<1	<1	<1	<1	<1	<1
<i>Listeria monocytogenes</i>	<1	<1	<1	<1	<1	<1
<i>Salmonella seftenberg</i>	<1	<1	<1	<1	<1	<1
<i>Candida albicans</i>	<1	<1	<1	<1	<1	<1
<i>Pseudomonas aeruginosa</i>	<1	<1	<1	<1	<1	<1

4 Bar Vacuum On	Air Sampling	Plate 0.1 m	Plate 0.25 m	Plate 0.5 m	Plate 0.75 m	Plate 1.0 m
Organism	cfu/ 100litres	cfu/plate	cfu/plate	cfu/plate	cfu/plate	cfu/plate
<i>Clostridium difficile</i>	<1	<1	<1	<1	<1	<1
<i>Acinetobacter lwoffii</i>	<1	<1	<1	<1	<1	<1
<i>Mycobacterium gordonae</i>	<1	<1	<1	<1	<1	<1
<i>Aspergillus niger</i>	32	<1	<1	<1	<1	<1
<i>S. aureus</i> (MRSA)	<1	<1	<1	<1	<1	<1
<i>Ecoli</i> verotoxic 0157:H7	<1	<1	<1	<1	<1	<1
Group A Streptococci (mix)	<1	<1	<1	<1	<1	<1
<i>Bacillus cereus</i>	<1	<1	<1	<1	<1	<1
<i>Listeria monocytogenes</i>	<1	<1	<1	<1	<1	<1
<i>Salmonella seftenberg</i>	<1	<1	<1	<1	<1	<1
<i>Candida albicans</i>	<1	<1	<1	<1	<1	<1
<i>Pseudomonas aeruginosa</i>	<1	<1	<1	<1	<1	<1

9.0 Discussion

In this trial we have examined two steam cleaning devices , manufactured by Matrix Cleaning Systems Ltd under laboratory conditions. These trials were conducted in “dirty” conditions where the challenge organisms were present in high numbers and the matrix was presented as a dry film on a stainless steel surface.

The goals of the trial included assessment of environmental reduction of the microbiological challenge together with an understanding of the thermodynamics involved. Additionally we sought to gain some understanding of the impact of operating each device upon the environment peripheral to the cleaning area

Cleaning systems based on steam generation appear to work by the creation of constantly replaced pressurised cloud of hot saturated water vapour under a cleaning head. As a general model the impingement of this vapour and direct steam impact on to surfaces together with the physical action of the head brushes serve to solubilise organic and microbiological materials.

With earlier generations of steam cleaning devices it was anticipated that the model proposed above would provide a regime capable of satisfactory levels of antimicrobial sanitisation. However, common criticisms of devices not offering the vacuum option involved the possible generation of microbiologically contaminated aerosols and sub lethal thermal dosing of organism. Our data suggests that under the conditions of trial no measurable, pressure generated cross contamination of surfaces outside the treatment area were occurred . (Table 6). We did record low levels of atmospheric recovery of Aspergillus niger however this organism is know to be present in the normal atmosphere of the test environment.

We believe there is strong evidence to suggest that the introduction of the vacuum feature in the Matrix devices strategically overcomes the limitations of similar surface sanitising devices which rely solely on steam doses.

Our results indicated the range of operational vent temperatures achieved, by the Matrix devices studied, range between 99.3 ‘C and 99.7 ‘c. These latter data correspond to the generation of core vapour temperatures ranging from 96.2 ‘C to 98.2 ‘C. Although we were unable to measure dwell time of microbiological debris in the vapour cloud nor assess any protective effect due to lecithin present, it is not unreasonable , assuming a dwell time of 0.1 seconds or greater, to postulate that any vegetative cells (other than those of extreme thermophilic organisms or some bacterial spores) present within the cloud would be inactivated with a high degree of efficiency.

During the generation of the steam and vacuum effect we have recorded treatment surface temperature maxima of circa 80 ‘c under standard conditions of use with the temperature minimally falling to circa 62 ‘C within 5 seconds after the dosage head had passed any single measurement point. Overall we assessed the time of dosing any of single surface treatment point at a temperature above 72‘C to be minimally circa 11 seconds and circa 6 seconds for temperatures at or above 80‘c. Again conditions likely afford Pasteurisation of non thermophilic vegetative cells and bacterial spores.

Considering the observed thermal performance data, we postulated that surface treatment trials would be successful in the removal of all vegetative microbial forms (and fungal spores) employed as challenge organisms during these trials. Our data (tables 3 & 4) confirms this prediction where in a greater than 99.999 % removal of all vegetative cells was repeatedly recorded at the treatment surface. However it is commonly accepted that the spores of Clostridium difficile under optimum conditions of treatment require exposure to conditions of 80‘C for a period 10 minutes to achieve satisfactory levels of log reduction. Our data suggests that > 99.999 spore removal was achieved by each device even though neither the 8 bar or 4 bar device under standard conditions of use would deliver a thermal treatment equivalent to 80‘c for 10 minutes.

With respect to the performance observed . in the environmental removal of *Clostridium difficile* spores we suggest that, not withholding any degree of thermal lethality, the contribution of the nebulisation of spores by the effects of steam vapour, coupled with that of vacuum removal into a sanitising solution , is a distinguishing key operational characteristic of the Matrix devices evaluated during this trial.

These observations are corroborated by the work conducted on the microbiological status of the debris bath (Table 5) where in our data clearly shows survival and the development of populations of some challenge organisms (including *Clostridium difficile*) in the debris reservoir in the absence of an effective biocide. Relatedly our data suggests that both antimicrobial agents studied were capable of satisfactorily maintaining an acceptable level of sanitation in the debris reservoir.

10.0 Conclusions

Within the conditions and constraints of this laboratory trial both the 8 Bar and the 4 Bar have met the criteria assigned for successful performance.

In these trials both devices consistently achieved a visually clean post treatment surface with > 99.999 % removal of all challenge organisms under dirty conditions. All test data indicated that the antimicrobial treatments applied to the debris reservoir achieved satisfactory performance

These objectives were achieved with no measurable level of microbiological contamination of peritreatment surfaces or the immediate atmosphere.

The data obtained robustly supports the premise that steam cleaning combined with vacuum removal is an effective technology.

On the basis of the results reported we are pleased to recommend both devices as candidates for flat surface cleaning solutions in both the medical and industrial environment.

It anticipated that equivalent levels of performance will be obtained in imminent Clinical and Industrial trials which will afford the opportunity for cross laboratory verification and further understanding of this cleaning strategy.



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