STUDY REPORT

STUDY TITLE

MEASURING THE EFFICIENCY OF TRUVOX STEAM CLEANER TO REMOVE DRIED MICROBIAL CONTAMINATION FROM A STAINLESS-STEEL SURFACE

STUDY REF: REF/PRO/BLT28

TYPE/ CODE: PRO

CUSTOMER

Truvox International

Unit C (East), Hamilton Business Park, Manaton Way, Botley Road, Hedge End, Southampton, SO30 2JR.

TEST FACILITY

BIOLABTESTS LTD.

3 Parade Court, Central Boulevard, Prologis Park, Coventry, UK. CV6 4QL.

Table of contents

1. **Study details**
   - 1.1 General
   - 1.2 Study responsibilities
   - 1.3 Study schedule
2. **Study objectives**
3. **Samples**
4. **Procedure**
5. **Observation and results**
   - 5.1 Raw data
   - 5.2 Percentage recovery
   - 5.3 Final recovery (a)
   - 5.4 Final recovery (b)
6. **Descriptive analysis**

1. Study details

1.1 General

Study title: Measuring the efficiency of Truvox product to remove dried microbial contamination from a stainless-steel surface.

Study reference: REF/PRO/BLT28

Test facility: BioLabTests Ltd., 3 Parade Court, Central Boulevard, Prologis Park, Coventry, CV6 4QL.

1.2 Study responsibilities

Study Conductor: Mr. Reynold Mpofu; Quality Officer, Mr. Richard Smith; Laboratory Manager.

Customer: Jonathan Clarke Truvox international Unit C (East), Hamilton Business Park, Manaton Way, Botley Road, Hedge End, Southampton, SO30 2JR, UK.

1.3 Study schedule

Experiment initiation date: 10.01.2023

Experiment end date: 11.01.2023

Study completion date: 13.01.2023

Date reported: 25.01.2023

2. Study objectives

To quantify what proportion of bacteria are removed from a stainless-steel surface, by the product under test using a standard pushing action of that product, from the known number of bacterial cells on that surface.

3. Samples

A box of unused and unopened, Truvox steamer cleaner was provided for testing.

Table 1: Sample description

Product name	Remarks
Truvox Steam Cleaner	Requested test wet against *Staphylococcus aureus*, *Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli*

4. Procedure

Strains of *Staphylococcus aureus* (ATCC 6538P), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 8739) were cultured separately to late log phase by overnight growth at 35˚C on Plate Count Agar (PCA). The bacteria were serially diluted in phosphate buffer solution (PBS) and measured using a spectrophotometer to obtain the number of colony forming units (CFUs) per ml and enumerated using the plate count method.

A flat surface of stainless-steel (approx. 500cm x 500cm) was cleaned and sterilised with 70% ethanol.

Cells were inoculated onto the sterile stainless steel in 10ml volumes of PBS; 9.25 x 10^5 CFUs of *S. aureus*, 9.78 x 10^5 CFUs of *E. coli,* 9.60 *x* 10^5 CFUs of *P. aeruginosa and* 6.87 *x* 10^5 CFUs of *B. subtilis*. The inoculum was spread over a surface of stainless-steel approximately 15cm x 15cm in size using a disposable spreader and allowed to air dry for 1 hour.

To assess the efficiency of the study to recover dried cells from the stainless-steel, areas of stainless-steel 1cm^2 in surface area were swabbed using a PBS-moistened cotton wool tip transport swab. The swab head was immediately transferred aseptically to 1ml of sterile PBS in an Eppendorf tube and vortexed for 60 seconds to suspend cells into the PBS. Suspensions of cells recovered from swabs were centrifuged at 13,000 rpm for 120 seconds to deposit the cells and resuspended in 1ml of sterile PBS for enumeration by a serial dilution and plate count method. This process was performed in triplicate using 3 randomly allocated 1cm^2 areas of inoculated stainless-steel for each bacterium, with mean values calculated.

Bacteria were removed from the stainless-steel surface using the test steam cleaner. The cleaning action implemented was to wait for the steamer to stop leaking after turning it on; move the steamer once across the stainless steel inoculated with the dry bacterial organisms.

Cells remaining on the stainless-steel surface were recovered and enumerated as described above. Suspensions of cells recovered from swabs were centrifuged at 13,000 rpm for 120 seconds to deposit the cells and resuspended in 1ml of sterile PBS for enumeration by plating onto PCA. Mean values were generated from the raw data and presented in the study results.

5. Observation and results

5.1 Raw data

The table below shows the colonies counted pre- and post-clean from swabs of 1cm^2 swatches for steam cleaning product.

Steam Cleaner

Table 2: Bacterial colony counts in triplicate and mean colony count pre- and post-clean for Steam cleaner.

	Bacteria	**Colony counts (CFUs)**			**Mean colony count (CFUs)**
Initial results pre-clean	*S. aureus*	36800	29100	20500	2.59 x 10^5
	E. coli	21900	41600	64000	4.25 x 10^5
	P. aeruginosa	12000	15900	19200	1.57 x 10^5
	B. subtilis	15200	9100	7700	1.07 x 10^5
Initial results post-clean	*S. aureus*	<1	<1	<1	1.00 x 10^1
	E. coli	<1	<1	<1	1.00 x 10^1
	P. aeruginosa	<1	<1	<1	1.00 x 10^1
	B. subtilis	<1	<1	<1	1.00 x 10^1

The number of bacteria recovered post-clean was significantly less than the number of bacteria recovered one hour after drying, pre-clean. This suggests that the steam cleaner is successful at removing large amounts of bacteria on a stainless-steel surface after single run through.

5.2 Percentage recovery

The percentage recovery determines the number of bacteria that were recovered after initial swabbing of 1cm^2 areas in triplicate before cleaning, following the one-hour dry. This will account for any cell death that has occurred between the initial inoculation and post-dry.

Steam Cleaner

Table 3: Efficiency of recovery of dried cells from stainless-steel from initial.

Bacteria	Number of cells initially inoculated on SS*	CFU recovered after 1 hour (pre-clean)	% Recovery
S. aureus	9.25 x 10^5	2.59 x 10^5	28.05%
E. coli	9.78 x 10^5	4.25 x 10^5	43.47%
P. aeruginosa	9.60 x 10^5	1.57 x 10^5	16.37%
B. subtilis	6.87 x 10^5	1.07 x 10^5	15.53%

*SS = stainless steel

5.3 Final reduction (a); calculated from cells initially inoculated on stainless-steel.

The table below determines the number of bacteria recovered post-clean. The results show the percentage reduction; this has *not* factored in natural cell death upon the one-hour dry.

Steam Cleaner

Table 4: Percentage reduction of dried cells from stainless steel post-clean against initial inoculation (a)

Bacteria	Number of cells initially inoculated on SS	CFU recovered after 1 hour (post-clean)	% Reduction
S. aureus	9.25 x 10^5	1.00 x 10^1	99.998%
E. coli	9.78 x 10^5	1.00 x 10^1	99.999%
P. aeruginosa	9.50 x 10^5	1.00 x 10^1	99.999%
B. subtilis	6.87 x 10^5	1.00 x 10^1	99.999%

5.4 Final reduction (b); calculated from cells recovered following the one-hour dry.

The final reduction after the one-hour dry factors in the reduction based on the recovery of bacteria that survived the one hour drying method. The results have been calculated taking into consideration natural cell death upon drying for one hour.

Steam Cleaner

Table 5: Percentage reduction of dried cells from stainless steel post-clean after inoculum dried (b)

Bacteria	CFU recovered after 1 hour (pre-clean)	CFU recovered after 1 hour (post-clean)	% Reduction
S. aureus	2.59×10^5	1.00×10^1	99.996%
E. coli	4.25×10^5	1.00×10^1	99.998%
P. aeruginosa	1.57×10^5	1.00×10^1	99.994%
B. subtilis	1.07×10^5	1.00×10^1	99.991%

Please note: the pass/failure rate is determined by the criteria set by the customer and their associates and not by BioLabTests.

6. Descriptive analysis

The results from section 5.3 have been calculated to show the percentage reduction which has been taken from the initial number of bacteria originally added to the stainless steel. Whilst this gives a good indication of the number of bacteria that were removed by the microfibre cloth against how much went on the stainless steel, it does not factor in the number of cells that naturally die during the one hour drying process.

Results from section 5.4 do factor in cell death upon the drying process. The percentage reduction was calculated from the number of bacteria recovered from the 1cm^2 swatches after one hour in triplicate.

This gives an indication of the number of bacteria that are still viable after one hour and a percentage reduction is determined from this value.

Although many bacteria will die rapidly within an environment with very little nutrients, other bacteria have developed the ability to survive on surfaces that would otherwise be considered unsuitable. Such environments include windowsills, sinks, cabinets or any other environment where the availability of rich nutrients is limited.
Due to the survival rate of certain bacteria within these environments, this can cause contamination issues for certain industries where hygiene is paramount. Pathogens such as *Campylobacter* and *Salmonella* can survive for up to 4 hours on surfaces whilst other bacteria usually dominated by the Gram-positive genera, such as *Staphylococcus* and *Streptococcus*, can survive for extended periods of time within dust particles.

Therefore, results taken from section 5.4 resemble a much more realistic approach to testing. The viable bacteria that withstood the drying process simulate the bacteria that are capable of living on surfaces for extended periods.

Section 5.2 demonstrates the efficiency of the recovery of dried cells from stainless steel pre-wipe. For example, of the 9.78 x 10^5 CFUs of *E. coli* that were applied to the stainless-steel and left to dry, 43.47%, or 4.25 x 10^5 CFUs were recovered following the initial swabs for the steam cleaner. The percentage reduction was calculated from the number of cells that were recovered (1.00 x 10^1), which gave a reduction of 99.99% post-clean. The cleaning action of the steam cleaner, as specified in section 4.0, was sufficient in removing the majority of *E. coli* cells from the surface. Similarly, for *S. aureus*, the cleaning action gave a 99.99% reduction of cells post-clean with an initial recovery of 28.05% or 2.59 x 10^5. The same action on *P. aeruginosa* resulted in a 99.99% reduction of cells post-clean from 16.37% or 1.07 x 10^5 that was recovered from the initial swabs and *B. subtilis* also resulted in a 99.99% reduction of cells from 15.53% or 1.07 x 10^5 that was recovered from the initial swabs.

Results that were calculated against the initial gave similar results to that of which were calculated from the post-dry recovery (pre-clean), giving a percentage reduction of 99.99% against both organisms (pre-clean).

Overall, the product Truvox Steam Cleaner was successful in removing large numbers of *S. aureus*, *E. coli, P. aeruginosa* and B. subtilis within laboratory settings, under wet conditions on a stainless-steel surface.

Reynold Mpofu (BSc Hons)
Microbiologist/Quality Officer
BioLabTests Ltd

Richard Smith (BSc Hons)
Laboratory Manager
BioLabTests Ltd

END OF REPORT