JLA's OTEX Processes
Report on the Fifth Validation Trial

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TECHNICAL *matters...*

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Executive Summary

The eight OTEX wash processes were investigated using a cost effective statistical experimental design. It was possible to validate the OTEX system by demonstrating that over the range of processing conditions used in the programmes there was a generally high and acceptable removal of micro-organisms from textiles (about a Log_{10} 6 reduction). This was sufficiently consistent to conclude that the system was both robust and effective.

The negligible counts of viable organisms found in general in wash liquors and on sterile swatches placed in the wash processes during the investigation supported the view that the process conditions were effective at disinfecting, rather than simply mechanically removing micro-organisms.

It was recommended that, since the implications for infection control are so important, validation study should be repeated at a second installation with the assistance of another independent microbiologist in order to verify the findings.

JLA's OTEX Processes; report on the fifth validation trial

1. Introduction

The OTEX process is based on an innovative means of introducing ozone into the washing process, primarily to provide a disinfecting action. The process is conducted at lower temperatures than normal OPL or industrial washing thereby offering savings in energy. It also offers other advantages such as lower detergent usage, leading to less water usage in rinsing. These opportunities make it very attractive for laundering patients' clothing in nursing and residential homes.

The purpose of this work was to validate the disinfecting action of the OTEX wash processes, of which there are eight at the time of reporting. These processes are intended to cover the different wash processes required in nursing homes etc.

2. Methodology

2.1 Validation trial

Validation requires that the key variables for micro-organism kill are statistically identified and their effect on micro-organism kill quantified. If this is conducted through a balanced statistical experimental design it should result in a regression equation which effectively models the process. This gives a full understanding of what is happening in the process and, very importantly, it provides the means of estimating what will happen to micro-organism kill if there are small changes to the key variables. This in turn allows an evaluation of the robustness of the process.

However to allow this type of modelling the microbial challenge must be so great that it will allow survivors from even the most severe treatment – otherwise there won't be any numbers with which to do the analysis.

Starting with this principle an experiment was designed to cover all the different OTEX processes in terms of the process (independent) variables which JLA believed would be important. Because there were nine variables a Plackett-Burman design was used in order to reduce the number of treatments to a manageable and economic level. PB designs should only really be used where it is known that only the key variables will have a significant effect and any interactions between them can be ignored.

This will almost certainly NOT be the case in a wash process, but there were two other considerations which made the choice reasonable.

i) The design would ensure that all the processing 'space' for the OTEX programmes would be investigated (with 3 variables the space would be 3D i.e. a cube – only the computer can handle 9D space!)

ii) JLA's expectation was that all micro-organisms would be killed, thereby validating all the processes at a stroke. Alternatively, using high challenges, it would be possible to quantify a minimum figure for micro-organism kill, which would satisfy any criteria e.g. at least a log5 kill might be achieved for every organism assessed.

2.2 Trial design

Given the considerations above the design shown in Annex A – Table A1 was selected with the aid of the statistical package Minitab version 12. It shows the nine independent variables chosen for investigation together with their symbols, selected ranges and the experimental design matrix. Mean results for Log_{10} Reduction (LRed of micro-organisms) are also shown for the four micro-organisms evaluated. As a check that the design was completely balanced the correlation matrix was examined Annex A – Table A2. All off diagonal values were zeroes showing that none of the independent variables in the design were correlated with each other and therefore were capable of independent estimation.

A randomised treatment plan for sequencing the trial was produced. This was needed so that each treatment condition would need to be set up afresh thereby reducing the chances of nuisance relationships occurring through e.g. correlation of increasing wash severity over the time taken to carry out the treatments.

2.3 Materials

2.3.1 Inoculated samples

In order to reflect the practical situation of laundering contracts from nursing homes etc. it was decided that test samples should be prepared by inoculation onto food stains because of the known protective effect of such stains and of textiles in general. The micro-organisms chosen were *E. coli, E. hirae, S. aureus and Cl. diff,* to give a loading of ~10^{10} organisms/cm^2.

For details on the preparation of inoculated samples, recovery of viable organisms, enumeration and treatment of raw data the reader is referred to Des O'Connor**.**

2.3.2 Artificially soiled monitors

Standard EMPA test monitors were used to vary the level of soiling in the load. No remission results have been provided so no analysis of the general soiling removal power of the process could be assessed.

2.4.3 Test loads

The test loads comprised polycotton laboratory coats.

2.4 Experimental

The 12 treatments required by the design were carried out in a microprocessor-programmable HW94 washer extractor machine (nominal capacity 9 kg) fitted with an OTEX ozone generator capable of variations in operation to meet the requirements of the design.

Replicated inoculated test pieces were placed in the pockets of laboratory coats used as ballast for the wash treatments. The test pieces were recovered into sealed, sterilised bags and sent for viable micro-organism recovery and enumeration. All procedures followed good microbiological practice.

EMPA test pieces to provide the required levels of soiling for each treatment were fixed to laboratory coats using plastic staples.

Wash liquor samples were collected to determine if viable micro-organisms were present and if dilution was a contributory factor to removal. Sterile fabric samples were also attached to laboratory coats in the load in order to determine if any such micro-organisms would be picked up from the wash liquor and distributed over the textiles, a form of dilution.

The treatments were carried out in random order and assigned corresponding codes so that the microbiologist analysing the samples could not relate them to specific treatments (blind)

3. Treatment of results

3.1 Reporting of data

Results of the recovery of viable organisms were reported to the author from all the replicate inoculated swatches and mean figures for each micro-organism in each treatment were calculated. The mean LRed results for the four micro-organisms evaluated are presented in Annex A – Table A1

Results for wash liquor and sterile swatch are shown in Annex A – Table A3

3.2 Treatment of data

Inspection of the means in Annex A – Table A1 suggested that the results for B70 and B82 for *E.coli* and *S. aureus* respectively could be outliers, but a statistical check showed that they should be included (see Annex B). However, inspection of the replicates for all organisms in run B70 revealed a very untypical variation (standard deviation of >3) in the results compared with all other treatment runs.

In general, it was clear from the spread of treatment means for each organism that there was insufficient variation to justify a full regression analysis of the treatment results.

4. Discussion of results

4.1 General nature of the data

Only for *E.coli* were organisms completely removed in the majority of treatments (Annex A – Table A1). For *E. hirae, S. aureus and Cl. Diff* LReds in the range 5 to 7 were found depending on the organism. The replicate results for all micro-organisms in run B70 were suspect because of the large standard deviations compared to other treatments.

Otherwise the lack of variation between treatments for all the organisms did not justify a detailed statistical analysis. This did not mean that the trial had been unsuccessful. Section 2 above described the alternative forms which validation could take and the results obtained qualified for the type of validation described in 2.1 ii).

All the programme conditions possible within the processing 'space' described by the nine key variables were accounted for by the statistical design used. Since the LReds achieved were consistently high it is therefore reasonable to conclude that the system and programmes offered are both robust and effective at removing micro-organisms from textiles under low temperature conditions.

In general there were only negligible counts for viable micro-organisms in the wash liquors and on the sterile swatches, broadly confirming that the treatments were effectively killing the microbial challenges, rather than the wash processes simply providing dilution. The one exception was for run B71 where higher, though not serious, counts were obtained. Unfortunately there were no data for the sterile swatches in this run (nor for runs B70 & B73)

5. Conclusion

5.1 The validation procedure was of the type described in 2.1 ii) above i.e. a generally high and acceptable LRed was achieved, demonstrating that the programmes overall were both robust and effective at removing micro-organisms from textiles.

5.2 The negligible counts of viable organisms found in general in wash liquors and on sterile swatches supported the view that the process conditions were effective at disinfecting at about the Log_{10} 6 reduction level, whether on the textiles or in the wash liquor.

6. Recommendations

Since the implications for infection control are so important, it would be advisable to repeat this validation study at a second installation with the assistance of another independent microbiologist in order to verify the findings.

Annex A Table A1. Details of the trial design and mean LRed results

		Main dip	Detergent Conc.	Load ratio	Soiling	2nd rinse duration	Oxygen pressure	Ozone conc.	Ozone flow rate	Rinse dip				
Units		cm	ml/k	kg	text	psi	leds	SCFH	cm	mins				
Batch	-1	11	5	5	No	3	2	1	15	5	Mean LRed results			
Ref	1	19	15	9	Yes	7	6	5	23	11				
		ML	D	L	S	R	OP	OC	OF	RL	*E. coli*	*S. aureus*	*E. hirae*	*Cl. diff*
B80	1	1	-1	1	1	-1	-1	1	-1	-1	10.7	6.1	8.0	7.2
B75	2	-1	1	1	-1	1	1	-1	-1	-1	10.8	5.5	6.5	6.2
B70	3	-1	-1	-1	-1	-1	-1	-1	-1	-1	7.6	7.4	7.0	7.3
B82	4	-1	-1	1	1	-1	1	-1	1	1	10.6	4.0	6.1	5.5
B76	5	1	-1	-1	-1	1	1	1	1	-1	10.8	6.3	7.6	6.9
B85	6	-1	1	-1	-1	-1	-1	1	1	1	10.7	6.4	6.6	7.6
B71	7	-1	-1	-1	1	1	1	1	-1	1	10.2	5.4	6.6	6.2
B84	8	1	1	-1	1	-1	1	-1	1	-1	10.7	5.5	5.9	6.9
B77	9	-1	1	1	1	1	-1	1	1	-1	10.8	6.0	7.1	6.8
B73	10	1	1	1	-1	-1	1	1	-1	1	9.0	5.8	7.6	5.7
B83	11	1	-1	1	-1	1	-1	-1	1	1	10.6	5.2	6.0	6.0
B78	12	1	1	-1	1	1	-1	-1	-1	1	10.8	5.6	6.3	6.3

Annex A Table A2. Correlation matrix

Independent variable correlation matrix

	M	*D*	*L*	*S*	*OP*	*OC*	*OF*	*RL*	*R*
M	1								
D	0	1							
L	0	0	1						
S	0	0	0	1					
OP	0	0	0	0	1				
OC	0	0	0	0	0	1			
OF	0	0	0	0	0	0	1		
RL	0	0	0	0	0	0	0	1	
R	0	0	0	0	0	0	0	0	1

All off-diagonal correlations are zero showing that the effects of the independent variables can be estimated exclusively i.e. not confounded with each other

Annex A Table A3. Wash liquor and sterile swatch results

Run Number	Type of sample	Mean counts (WL cfu/ml; SS cfu/dm^2)			
		E. coli	*E. hirae*	*S. aureus*	*Cl. diff*
B80	WL	0	0	0	<1
	SS	0	0	0	0
B75	WL	0	0	1	1
	SS	0	0	<1	<1
B70	WL	0	3	3	1
	SS	n/a	n/a	n/a	n/a
B82	WL	0	0	0	2
	SS	0	10	11	1
B76	WL	0	0	0	0
	SS	0	0	0	0
B85	WL	0	0	0	0
	SS	0	0	0	0
B71	WL	<1	7	21	16
	SS	n/a	n/a	n/a	n/a
B84	WL	0	0	0	<1
	SS	0	0	0	0
B77	WL	0	0	<1	0
	SS	0	0	<1	<1
B73	WL	0	1	<1	3
	SS	n/a	n/a	n/a	n/a
B83	WL	0	0	0	1
	SS	0	0	1	1
B78	WL	0	0	0	2
	SS	0	0	0	0

WL = wash liquor SS = sterile swatch

Annex B Detection of outliers

Outliers are results which belong to a distribution with a different mean and standard deviation from the rest of the results. There are some sophisticated tests for detecting outliers, but in most cases the following simple test will suffice.

As an example consider the set of means for *E. coli*.

10.7, 10.8, 7.6, 10.6, 10.8, 10.7, 10.2, 10.7, 10.8, 9.0, 10.6, 10.8

Inspection suggests that possibly 7.6 is an outlier. The following equation may be used to give some statistical justification to rejecting 7.6 as an outlier

If (HV – LV)/(HV – NL) > 2, then LV is probably an outlier

Where HV = highest value, LV = lowest value and NL = next lowest value

Thus (10.8 – 7.6)/(10.8 – 9.0) = 1.78, so 7.6 is not likely to be an outlier

Similarly for *S. aureus* the value 4.0 is not an outlier with a test statistic of 1.55

For completeness outliers which are greater than the other results can similarly be tested using the equation If (HV – LV)/(NH – LV) >2 HV is probably an outlier, where NH is the next highest