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Confidential report for:

Medical Wire & Equipment co Ltd
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Report on:

Pre-validation study of a novel device able to detect *Salmonella* spp.

Work performed by Campden BRI (Chipping Campden) Limited
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1. INTRODUCTION

Medical Wire & Equipment co. Ltd develops and manufactures hygiene monitoring systems based on swabs. They have recently developed a system designed to enrich and detect *Salmonella* spp. and wished to have a third-party verify the specificity of this device via a small preliminary inclusivity / exclusivity study.

Campden BRI (CC) were requested to investigate the specificity of the novel Salmonella specific device via a preliminary inclusivity / exclusivity study, if successful this would lead to a full inclusivity/exclusivity study.

2. METHODS

2.1 Products

The products tested are shown below along with the sample code.

Table 1: Products

Clients product	Sample code
Salmonella Transwabs	MB/134198/1-6

The samples were received on 28/08/14 and were in satisfactory condition.

All samples were labelled with appropriate sample code.

Samples were stored at ambient conditions prior to testing.

Testing was carried out between 23/09/14 and 25/09/14.

2.2 Organisms

The following microbial strains were used in this trial.

Table 2: Organisms for the exclusivity study

Number	Organism	Campden code	Source	Media for culture growth
134198/1	E. coli	CRA 2003	Fish	NB
134198/2	S. aureus	CRA 1224	Margarine	TSB

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Table 3: Salmonella strains for used in the inclusivity study

Sample code	Organism	Campden code	Source	Media for culture growth
134198/3	*Salmonella* Enteritidis	CRA 1004	Chicken	NB
134198/4	*Salmonella* Enteritidis	CRA 3505	Fish cakes	NB
134198/5	*Salmonella* Enteritidis	CRA 1944	Chicken	NB
134198/6	*Salmonella* Typhimurium	CRA 11634	Bovine ATCC 14028	NB

The organisms in Tables 2 and 3 were taken from frozen beads stored in the Campden BRI culture collection. They were then grown in rich nutrient media detailed in Tables 1 and 2, and all cultures were incubated at 37 ± 1ºC overnight, with the aim to have at least $1x10^8$ colony forming units (cfu/ml) per ml of each culture, which was estimated via a microscopic count using a haemocytometer.

2.3 Sample Inoculation

For each organism listed in Table 3, the neat culture was diluted to a concentration of 10^5 and 10^4 cfu/ml in MRD. For each organism 100µl of each dilution (approximately 10^4 and 10^3 cells) was inoculated directly onto the swab part of separate *Salmonella* Transwab devices; the swab was placed into the tube. Incubated at 37°C for up to 48 hours, the appearance or not of a black colouration was recorded at 24 and then again at 48 hours.

This was repeated for the organisms listed in Table 2, although with only the higher concentration of cells 10^4, therefore approximately 10^3 cells in the 100µl sample.

Note: the final level in the samples could be up to 1 log unit above or below the target level although usually it will be within 0.5log units.

2.4 Microbiological/chemical analysis

The concentration of the inoculum for all organisms was evaluated using the following methods

Table 4: Microbiological tests

Organism	Test method	Method Summary*
Aerobic Plate count	TES-MB-002**	Pour plate with PCA. Incubation at 37 ± 1°C for 24± 2hr

* full details of tolerances on method time and temperatures are given in the full methods.
**Adapted method temperature and time changed from 30°C for 48hr to 37°C for 24 hr to reflect organisms used in trial.

3. RESULTS

3.1 Study Data

The microbiological and colour change data from this trial is given in Table 5 below.

Table 5: Results of Trial

Sample code	level code	Culture	campden culture code	level aim (on device)	PCA results		level of culture onto swab		Transwab			
				Log cfu	cfu/ml	log cfu/ml	cfu	log cfu	24 hr		48 hr	
										Colour		Colour
134198/1	a	*S. aureus*	CRA 1224	4	6.7E+04	4.8	6.7E+03	3.8	-	all red	-	all red
134198/2	a	*E. coli*	CRA 2003	4	6.1E+04	4.8	6.1E+03	3.8	-	all red	-	all red
134198/3	a	*Salmonella* Typhimurium	CRA 11634	4	8.7E+04	4.9	8.7E+03	3.9	+	all black	+	all black
	b			3			8.7E+02	2.9	+	black streak	+	all black
134198/4	a	*Salmonella* Enteritidis	CRA 1944	4	7.9E+04	4.9	7.9E+03	3.9	+	black streak	+	mostly black
	b			3			7.9E+02	2.9	-	all red	-	all red
134198/5	a	*Salmonella* Enteritidis	CRA 1004	4	1.1E+05	5.0	1.1E+04	4.0	+	black streak	+	all black
	b			3			1.1E+03	3.0	+	black streak	+	mostly black
134198/6	a	*Salmonella* Enteritidis	CRA 3505	4	4.7E+04	4.7	4.7E+03	3.7	+	black streak	+	mostly black
	b			3			4.7E+02	2.7	+	black streak	+	mostly black

3.2 Study criteria: Analysis of results and interpretation

The results show that the exclusivity cultures, *Staphylococcus aureus* and *Escherichia coli* showed no change in colour over the 48 hour period and as such gave a negative result for presumptive presence of *Salmonella*.

The four *Salmonella* cultures all produced a positive result at the higher concentrations of approximately 10^4 colonies by the 24 hour reading, and three of the four *Salmonella* cultures produced a positive result at the lower concentration of approximately 10^3 colonies by the 24 hour reading. One negative inclusivity result was observed, that was the *Salmonella* Enteritidis CRA 1944 at a level of 2.9 log colonies. The levels of cultures applied to the devices ranged between 3.7 and 4.0 log colonies for the higher concentration and between 2.7 and 3.0 log colonies for the lower concentration.

4. CONCLUSION

The data from this study has shown:

- Application of the exclusivity cultures at a concentration of 3.8 log produced negative results, indicating some selectivity.
- Application of the inclusivity cultures at a concentration of 3.7 – 4.0 log produced positive results, and application of the same cultures at a concentration of 2.7 – 3.0 log produced positive results in three out of the four *Salmonella* cultures.

This is a preliminary trial and further work needs to be carried out to determine the specifity of the device.

Note: These results are valid for batches of product produced and stored under identical conditions. Any changes in product formulation or storage conditions may change the results expected to be obtained.

Appendix: Glossary of Microbiological Media

Agar/broth	Full name	Media Manufacturers and Codes
MRD	Maximum Recovery Diluent	LabM LAB103 Oxoid CM0733
NB	Nutrient Broth	Oxoid CM0001
PCA	Plate Count Agar	LabM LAB149 Oxoid CM0325
TSB	Tryptone Soy Broth	CM0129