AMBIC®

STUDY REPORT

In-vitro Comparison of the Efficacy of Teat Disinfectants Applied as a Dip or as a Foam

AMBIC: A Healthy Herd • A Healthy Profit

STUDY REPORT

IAH STUDY REF: IAH B14

STUDY TITLE: ***In-vitro*** **comparison of the efficacy of teat disinfectants applied as a dip or as a foam**

STUDY LOCATION:
Institute for Animal Health
Compton, Nr. Newbury
Berkshire, RG20 7NN
UK

STUDY SPONSOR:
Ambic Equipment Limited
Avenue Four

Station Lane
Witney
Oxfordshire
OX28 4XT

DISTRIBUTION LIST:

Study Sponsor	x2 bound, x1 unbound, 1x signature page (1x bound & sig. page to return to IAH)
Investigator	x1 unbound
Contracts Group files	x1 unbound
Contracts Group archive	x1 bound
Consultant	x1 bound, if required

STUDY PROTOCOL: **Final version 13/05/2003**

STUDY DATES: **21/05/2003 – 23/05/2003**

KEY WORDS: **Teat disinfectant, bacterial kill, *in-vitro* study**

LIST of CONTENTS PAGE

SIGNATURE PAGE

The undersigned accepts this report on behalf of the Sponsor:

Signature: ..... Date: 30./6./03

Name (please print): ANDREW BEGGIN

Position: MANAGING DIRECTOR

The undersigned certify that, according to the best of their knowledge and ability, that this report is a complete and accurate description of the data generated during the study.

Investigator:

Signature: ..... Date: 25/06/03

Name: J Cooper BSc PhD

Position: Research Scientist

Report authorised by the Head of Contracts Group:

Signature: ..... Date: 26./6./03

Name: Mervyn R. Burrows FIMLS

Position: Head of Contracts Group

The Institute for Animal Health, Compton, carries out efficacy studies according to GCP standards (as endorsed by the GCP Working Group at Step 7 of the VICH* Process, June 2000) and relevant Parts of Directive 92/18/EEC. However, elements of GCPV are not appropriate to the IAH-Sponsor relationship and Appendix 1 clarifies the delegations of responsibility to the IAH from the Sponsor.

* *International Co-operation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH).*

The Sponsor was responsible for quality assurance monitoring. All raw data will be returned to the Sponsor for archiving at the end of the study.

PERSONNEL AT STUDY SITE

Investigator:
J Cooper BSc PhD

Assistant Investigator:
JA Clarke

IAH Study Administrator:
MM Carr BSc PhD DipRQA

Supervisor of Animal Husbandry:
P Collins AIBiol

Laboratory Procedures:
JA Clarke
H Davies BSc (Hons)
V Wilson

Study Consultant(s):
E Hillerton BSc PhD

Statistical Advice:
University of Reading

A list of other staff involved in the trial is included in the Trial Master file. Personnel involved in the trial were briefed on their responsibilities prior to commencement of the trial.

SUMMARY

In *in-vitro* test was used to investigate the relative efficacies of a teat-disinfectant when applied as either a foam or a liquid dip against a combination of three mastitis causing bacterial species (*Staphylococcus aureus*, *E.coli* and *Streptococcus uberis*). The test involved using strips of pig-skin (n=20) to imitate the skin surface of the udder. The strips were inoculated with bacterial cultures followed by treatment with either water, the foamed test product or the liquid test product. Following a standard exposure time the numbers of surviving bacteria were estimated.

Both the liquid and the foam were highly effective at reducing bacterial counts of all three species relative to the water treated control.

The product was more effective at reducing bacterial counts when applied as a foam than as a dip against two of the three species investigated (*S.aureus*, $P = 0.021$; *E.coli*, $P = 0.006$). Treatment with foam reduced *S. uberis* counts relative to treatment with disinfectant dip but not significantly ($P = 0.084$). Overall, logarithmic bacterial counts following treatment with foam were approximately 50% lower than following treatment with the liquid dip.

STUDY REPORT

AIM

To compare the efficacy of a teat dip against a combination of three bacterial species when applied as either a liquid dip or as a foam.

INTRODUCTION

Matters of quality assurance, study administration and contractual considerations are detailed in Appendix 1.

The customer wished to obtain data to support a marketing claim. The customer wished to show that a teat disinfectant is as effective in terms of bacterial kill when applied as a foam as it is when applied as a dip.

The effectiveness of two disinfectant preparations was compared using an *in-vitro* test which involved testing the disinfectants against bacterial preparations applied to pig skin. Strips of pig skin were used in this *in-vitro* test to mimic the skin surface of the udder. This allowed the experiment to be conducted under controlled laboratory conditions. The alternative would have been to conduct this study in lactating dairy cows. This would have been labour intensive and expensive. This alternative approach provided meaningful data without the need to use experimentally infected animals.

MATERIALS & METHODS

Study outline

Pigskin was obtained fresh from a carcass from a local butchers and stored at -20°C until required for use. Skin strips of approximately 8 cm x 3 cm, were cut and labelled. All subsequent handling of skin-strips was performed in a safety cabinet with a filtered airflow to prevent environmental contamination of the samples.

Skin strips were hung vertically and then sterilised by submersion in methylated spirit and left to dry at room temperature for approximately 20 minutes.

A known volume of a bacterial suspension (100µl), containing three mastitis causing species (*Staphylococcus aureus*, *E.coli* and *Streptococcus uberis*) at a known concentration was applied to each skin strip. The strips were left to dry at room temperature for approximately 20 minutes.

Strips were then hung vertically, briefly immersed in the test product and left to dry for a further 30 minutes. Test products were used as in Table 1.

Table 1. Treatments

Group	Bacteria applied?	Disinfectant	Sample size
1	Yes	Dip	20
2	Yes	Foam	20
3	Yes	Water	20
4	No	None	6

Strips were then dropped into Ringers solution and mixed to halt the action of the disinfectant.

Bacterial counts from each sample were obtained by making serial tenfold dilutions of the Ringers solution and culturing a known volume on to media selective for the particular bacteria under test. Plates were incubated and numbers of colonies counted to determine the numbers of bacteria present. As there were three bacterial species under investigation, duplicate plates were prepared of three different media for each dilution. Hence each dilution was plated out six times. To determine the effectiveness of each disinfectant treatment, bacterial counts after immersion in disinfectant were compared with water-treated controls.

The effectiveness of the product was examined over three discreet time periods to ensure a manageable number of samples were processed each day. During the first period six samples treated with water were processed along with seven treated with dip and seven treated with foam. During the second period seven samples of each of the three treatments were processed. During the third period seven samples treated with water were processed along with six treated with dip and six treated with foam. The effect of time period was incorporated into the subsequent statistical analysis. Six negative control samples were also processed (no bacteria or disinfectant applied) to validate the test system.

Product & application

Ready-to-Use (0.51% w/v iodine, 6% emollients; Kilco Chemicals) was supplied by the Sponsor (Batch number 030330, expiry date March 2004). The product was applied using a standard teat-dip cup for samples in Group 1 or using a modified foaming teat-dip cup connected to a compressed air-supply (as supplied by Sponsor) for samples in Group 2.

Bacterial cultures & administration

Pigskin was inoculated with a mixture of three mastitis causing bacteria species. These were *Staphylococcus aureus* (strain B14/1 (M60)), *E.coli* (strain B14/2 (P4)) and *Streptococcus uberis* (strain B14/3 (0140J)). All isolates were stored in Robinson's Cooked Meat Broth with 15% glycerol at –80°C prior to use and were strains known to produce mastitis in cattle.

Isolates were grown aerobically overnight in Todd Hewitt broth at approximately 37°C. Cultures were freshly prepared prior to each period of inoculations. Each isolate was grown separately to give an estimated target count of 1×10^7 cfu/ml. Cultures were then diluted to give an estimated 1×10^6 cfu/ml concentration and the three species combined prior to infection of skin samples. 100µl of the pooled inoculum was applied to each skin strip and spread using a sterile loop to cover as much of

the surface as possible. Bacterial counts were performed on each culture prior to them being diluted and pooled.

Laboratory Procedures

The skin strips were then treated with dip, foam or water by immersion using a teat cup supplied by the sponsor. Strips were dipped then left for a 30-minute exposure period. The strips were then placed in to a 9ml volume of Ringers solution and vortexed for 30 seconds to stop the action of the disinfectant.

Serial tenfold dilutions were made of the vortexed skin strips in Ringers solution in PBSa and were plated onto Columbia + Strep supplement, blood agar and McConkey agar to culture *S.uberis*, *S.aureus* and *E.coli* respectively. Plates were incubated overnight at 37°C. Colonies were identified on the basis of colonial morphology.

Counts were performed for each of the three bacterial species on each sample. The bacterial count was taken as an average of the duplicate plates at the dilution showing the highest number of countable colonies. The duplicate plates that were used to determine the count were those of the most concentrated dilution that gave a meaningful result. The test system only allowed for plates yielding bacterial counts greater or equal to 1×10^1 cfu / ml to be detected i.e. 1 colony from the skin strip in 9ml of ringers solution. Hence plates that yielded counts of less that 1×10^1 cfu / ml were allocated a bacterial count of zero.

Statistical methods & assessment

Formal statistical analysis was performed using the statistical software package GenStat Release 6.1.

Bacterial counts were log transformed prior to analysis. The actual transformation used was log10(count +1). Use of this formula instead of log10(count) allow for values of zero to be included in the analysis.

Bacterial counts from skin strips treated with foamed disinfectant were compared to those treated with a disinfectant dip using ANOVA with a block structure to take into account the effect of the different time periods during which the strips were processed.

Standard errors were calculated by using the following formula:

$$\text{Standard error of the mean} = \frac{\text{standard deviation of the mean}}{\sqrt{\text{sample size}}}$$

RESULTS

Bacterial cultures

Bacterial counts of the three undiluted cultures that were used to produce the inoculum that was applied to the skin strips are given in Table 2.

Table 2. Bacterial counts of neat cultures used to prepare the inoculum

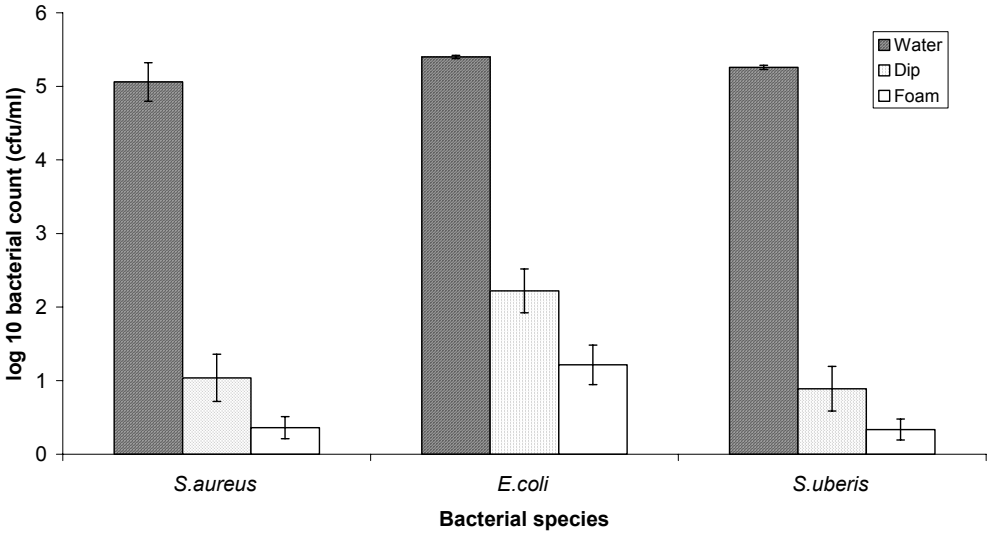
Time period	Bacterial count (cfu/ml)		
	S. aureus	*E.coli*	*S.uberis*
1	2.35×10^7	7.5×10^7	2.9×10^7
2	3×10^7	5.5×10^7	4.5×10^7
3	3×10^7	4.2×10^7	2.5×10^7

Counts were similar for all species on all days and were close to the target count of 1×10^7.

Bacterial counts following treatment

Bacterial counts obtained from each sample following treatment are presented in Appendix 2. Means of logarithmically transformed bacterial counts for each treatment against each bacterial species are summarised in Figure 1.

Figure 1. Bacterial counts following treatment with either water or a disinfectant applied as either a dip or a foam



Both dip and foam treatments resulted in a marked reduction in bacterial counts for all three species tested relative to the water treated controls (Figure 1).

Numbers of bacteria isolated from water-treated controls were fairly similar, regardless of bacterial species or time period (count range = 4.1×10^4 to 1.1×10^5). Treatment with the disinfectant applied as a dip reduced the numbers of bacteria but there was quite a variation in counts (count range = $<1 \times 10^1$ to 1.19×10^4) with the greatest variation in counts being shown for *E.coli*. Treatment with the disinfectant applied as a foam also reduced the numbers of bacteria but the range in the counts was not as great as with the dip (count range = $<1 \times 10^1$ to 4×10^3). Once again, the greatest variation in counts was shown for *E.coli*.

Analysis of variance showed that treatment of the samples with disinfectant foam resulted in significantly lower *S. aureus* counts than treatment with disinfectant dip (log transformed data: foam mean = 0.36, dip mean = 1.04; $F = 5.83$, $P = 0.021$). Treatment of the samples with disinfectant foam resulted in significantly lower *E. coli* counts than treatment with disinfectant dip (log transformed data: foam mean = 1.21, dip mean = 2.22; $F = 8.51$, $P = 0.006$). Treatment of the samples with disinfectant foam appeared to reduce *S. uberis* counts relative to treatment with disinfectant dip (Figure 1) however this difference was not statistically significant (foam mean = 0.33, dip mean = 0.89; $F = 3.16$, $P = 0.084$).

On average, logarithmic counts approximately 1.8, 2.3 and 1.8 times higher after treatment with dip than with foam for *S.aureus*, *E.coli* and *S.uberis* respectively (calculated by comparing logarithmic counts during each treatment period and calculating the average of the three periods).

DISCUSSION

Compared to water-treated controls, the product was highly effective in reducing counts of three mastitis causing bacteria species when applied as either a liquid dip or as a foam in an *in-vitro* test system involving pig-skin being inoculated with bacteria.

Under the test conditions, treatment with foam resulted in lower bacterial counts than treatment with liquid dip for all three bacterial species and a statistically significant difference was demonstrated for two of the three bacteria used. Overall, treatment with the foam resulted in an average decrease in logarithmic bacterial count of approximately 50% relative to the liquid dip treatment.

A smaller volume of the product is used when it is applied as a foam as opposed to being applied as a liquid dip. Hence there are economic benefits associated with use of the foamed product. The results of this *in-vitro* study suggest that such benefits would be achieved without compromising the effectiveness of the product.

CONCLUSIONS

During an *in-vitro* test, application of a foamed teat disinfectant was more effective in reducing bacterial counts on a skin surface of three mastitis causing species than when the same product was applied as a liquid dip.

APPENDIX 1 - GCPV for Experimental Studies – Clarification of Responsibilities

The IAH conduct efficacy studies using experimental disease models in the target species. These are conducted to GCP standards (as endorsed by the GCP Working Group at Step 7 of the VICH* Process, June 2000). However, since GCPV is designed primarily for the field situation, certain elements are not appropriate to the working practices of the IAH. Many of the responsibilities of the Sponsor may be delegated to the IAH acting as a Contract Research Organisation (CRO), as defined in Section 4.3 of GCPV guidelines.

This Appendix is designed to satisfy the requirement of Section 4.3.2 of GCPV, which states that 'any study-related duty or function that is delegated to a CRO should be specified in writing' and 'any study-related duties or functions not specifically delegated to a CRO are retained by the sponsor'.

❖ ❖ ❖

In addition to the Investigator responsibilities defined in GCPV, the IAH Investigator may assume some or all responsibility for the following aspects of the study (relevant GCPV section listed in parenthesis). However, it must be stressed that the ultimate responsibility for the quality & integrity of the study data always resides with the Sponsor.

- IAH SOPs will be used for the procedural and technical elements of the study unless agreed otherwise in advance of the study (section 4.2.5).
- The IAH will normally produce the study protocol using its own standard format, based on GCPV (section 4.2.6).
- IAH staff will be responsible for preparation of local DCFs (see section 4.2.8.2 for multicentre studies).
- IAH Investigator will ensure the proper disposal of all study animals *etc.* (Section 4.2.11).
- IAH Investigator will ensure proper & final disposal of product, feed *etc*. (Section 4.2.13).
- IAH will prepare the final report unless otherwise agreed with the Study Sponsor, to a format agreed between the Study Sponsor and the Investigator (section 4.2.15).
- IAH staff, or consultants, as agreed with the Study Sponsor, may provide advice on experimental design & perform statistical analyses of results.
- The IAH will usually undertake electronic data entry and data analyses. A properly validated computer programme will be used and the accuracy of transcription will be checked.
- IAH will ensure that personnel involved in the study have been adequately briefed on the details of the study.

* International Co-operation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH).

APPENDIX 2 – Bacterial counts

Group 1 – Water controls

Time period	Sample ID	*E.coli* Count	*E.coli* Log (10) count +1	*S. aureus* Count	*S. aureus* Log (10) count +1	*S.uberis* Count	*S.uberis* Log (10) count +1
1	51	2.71×10^5	5.433	4.10×10^4	4.613	1.38×10^5	5.140
	52	3.08×10^5	5.489	1.48×10^5	5.170	1.62×10^5	5.210
	62	2.71×10^5	5.433	1.00×10^5	5.000	1.38×10^5	5.140
	63	2.56×10^5	5.408	1.47×10^5	5.167	1.64×10^5	5.215
	64	2.46×10^5	5.391	1.20×10^5	5.079	1.92×10^5	5.283
	65	1.26×10^5	5.100	1.01×10^5	5.004	1.13×10^5	5.053
		Mean	**5.376**	**Mean**	**5.006**	**Mean**	**5.173**
		S.E.	**0.057**	**S.E.**	**0.084**	**S.E.**	**0.033**
2	68	2.37×10^5	5.375	1.17×10^5	5.068	2.10×10^5	5.322
	69	2.30×10^5	5.362	7.70×10^4	4.886	1.80×10^5	5.255
	70	3.45×10^5	5.538	1.30×10^5	5.114	2.63×10^5	5.420
	71	2.94×10^5	5.468	1.56×10^5	5.193	2.69×10^5	5.430
	86	3.12×10^5	5.494	1.92×10^5	5.283	2.75×10^5	5.439
	87	2.19×10^5	5.340	1.46×10^5	5.164	1.85×10^5	5.267
	88	2.16×10^5	5.334	1.31×10^5	5.117	1.88×10^5	5.274
		Mean	**5.420**	**Mean**	**5.118**	**Mean**	**5.344**
		S.E.	**0.031**	**S.E.**	**0.047**	**S.E.**	**0.031**
3	96	3.56×10^5	5.551	1.50×10^5	5.176	1.91×10^5	5.281
	97	2.23×10^5	5.348	1.69×10^5	5.228	1.07×10^5	5.029
	98	1.92×10^5	5.283	7.10×10^5	4.851	1.53×10^5	5.185
	106	2.73×10^5	5.436	5.90×10^4	4.771	2.24×10^5	5.350
	107	2.49×10^5	5.396	9.70×10^4	4.987	1.47×10^5	5.167
	108	2.57×10^5	5.410	1.60×10^5	5.204	2.93×10^5	5.467
	109	2.48×10^5	5.394	1.30×10^5	5.114	1.73×10^5	5.238
		Mean	**5.403**	**Mean**	**5.047**	**Mean**	**5.245**
		S.E.	**0.031**	**S.E.**	**0.068**	**S.E.**	**0.053**
		Overall Mean	**5.400**	**Overall Mean**	**5.060**	**Overall Mean**	**5.258**
		Overall S.E.	**0.027**	**Overall S.E.**	**0.261**	**Overall S.E.**	**0.027**

APPENDIX 2 – Bacterial counts (continued)

Group 1 – Disinfectant applied as a dip

Time period	Sample ID	*E.coli*		*S. aureus*		*S.uberis*	
		Count	Log (10) count +1	Count	Log (10) count +1	Count	Log (10) count +1
1	44	1.00×10^1	1.041	3.00×10^1	1.491	$< 1 \times 10^1$	0.000
	45	1.11×10^4	4.045	8.70×10^3	3.940	2.56×10^3	3.408
	46	7.80×10^3	3.892	4.40×10^3	3.644	1.40×10^3	3.146
	47	3.93×10^3	3.595	3.40×10^3	3.532	7.90×10^2	2.898
	48	2.00×10^1	1.322	3.00×10^1	1.491	4.00×10^1	1.613
	49	6.00×10^1	1.785	2.30×10^2	2.364	$< 1 \times 10^1$	0.000
	50	1.80×10^2	2.258	3.20×10^2	2.507	1.00×10^1	1.041
		Mean	**2.563**	**Mean**	**2.710**	**Mean**	**1.730**
		S.E.	**0.516**	**S.E.**	**0.384**	**S.E.**	**0.549**
2	72	1.19×10^4	4.076	$< 1 \times 10^1$	0.000	4.10×10^3	3.613
	73	1.00×10^1	1.041	6.00×10^1	1.785	$< 1 \times 10^1$	0.000
	74	2.00×10^1	1.322	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	75	3.10×10^2	2.493	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	76	3.40×10^3	3.532	$< 1 \times 10^1$	0.000	1.1×10^2	2.045
	77	2.70×10^3	3.432	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	78	4.30×10^3	3.634	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
		Mean	**2.790**	**Mean**	**0.2550**	**Mean**	**0.808**
		S.E.	**0.420**	**S.E.**	**0.2550**	**S.E.**	**0.549**
3	100	4.20×10^2	2.624	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	101	2.00×10^1	1.322	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	102	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	103	9.00×10^1	1.959	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	104	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	105	1.00×10^1	1.041	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
		Mean	**1.158**	**Mean**	**0.000**	**Mean**	**0.000**
		S.E.	**0.429**	**S.E.**	**0.000**	**S.E.**	**0.000**
		Overall Mean	**2.221**	**Overall Mean**	**1.038**	**Overall Mean**	**0.888**
		Overall S.E.	**0.297**	**Overall S.E.**	**0.321**	**Overall S.E.**	**0.304**

APPENDIX 2 – Bacterial counts (continued)

Group 3 – Disinfectant applied as a foam

Time period	Sample ID	*E.coli*		*S. aureus*		*S.uberis*	
		Count	Log (10) count +1	Count	Log (10) count +1	Count	Log (10) count +1
1	55	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	56	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	57	2.90×10^2	2.464	1.00×10^2	2.004	1.20×10^2	2.083
	58	2.00×10^1	1.322	$< 1 \times 10^1$	0.000	1.00×10^1	1.041
	59	1.00×10^1	1.041	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	60	1.00×10^1	1.041	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	61	5.00×10^1	1.708	6.00×10^1	1.785	$< 1 \times 10^1$	0.000
		Mean	**1.082**	**Mean**	**0.541**	**Mean**	**0.446**
		S.E.	**0.361**	**S.E.**	**0.350**	**S.E.**	**0.310**
2	79	1.07×10^3	3.030	2.00×10^1	1.322	1.00×10^1	1.041
	80	1.40×10^2	2.149	1.00×10^1	1.041	3.00×10^1	1.491
	81	8.00×10^1	1.908	1.00×10^1	1.041	$< 1 \times 10^1$	0.000
	82	5.90×10^2	2.772	$< 1 \times 10^1$	0.000	1.00×10^1	1.041
	83	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	84	1.00×10^1	1.041	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	85	4.00×10^3	3.602	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
		Mean	**2.072**	**Mean**	**0.486**	**Mean**	**0.511**
		S.E.	**0.466**	**S.E.**	**0.232**	**S.E.**	**0.247**
3	90	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	91	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	92	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	93	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	94	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
	95	1.60×10^2	2.207	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
		Mean	**0.368**	**Mean**	**0.000**	**Mean**	**0.000**
		S.E.	**0.368**	**S.E.**	**0.000**	**S.E.**	**0.000**
		Overall Mean	**1.214**	**Overall Mean**	**0.360**	**Overall Mean**	**0.335**
		Overall S.E.	**0.269**	**Overall S.E.**	**0.150**	**Overall S.E.**	**0.141**

APPENDIX 2 – Bacterial counts (continued)

Group 4 – Negative controls

Time Period	Sample ID	*E.coli*		*S. aureus*		*S.uberis*	
		Count	Log (10) count +1	Count	Log (10) count +1	Count	Log (10) count +1
1	54	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
1	66	Problem with sample. Results disregarded					
2	67	1.20×10^2	2.083	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
2	89	9.10×10^2	2.960	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
3	99	2.80×10^4	4.447	7.40×10^2	2.870	$< 1 \times 10^1$	0.000
3	110	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000	$< 1 \times 10^1$	0.000
		Mean	1.900	**Mean**	0.574	**Mean**	**0.000**
		S.E.	0.862	**S.E.**	0.574	**S.E.**	**0.000**