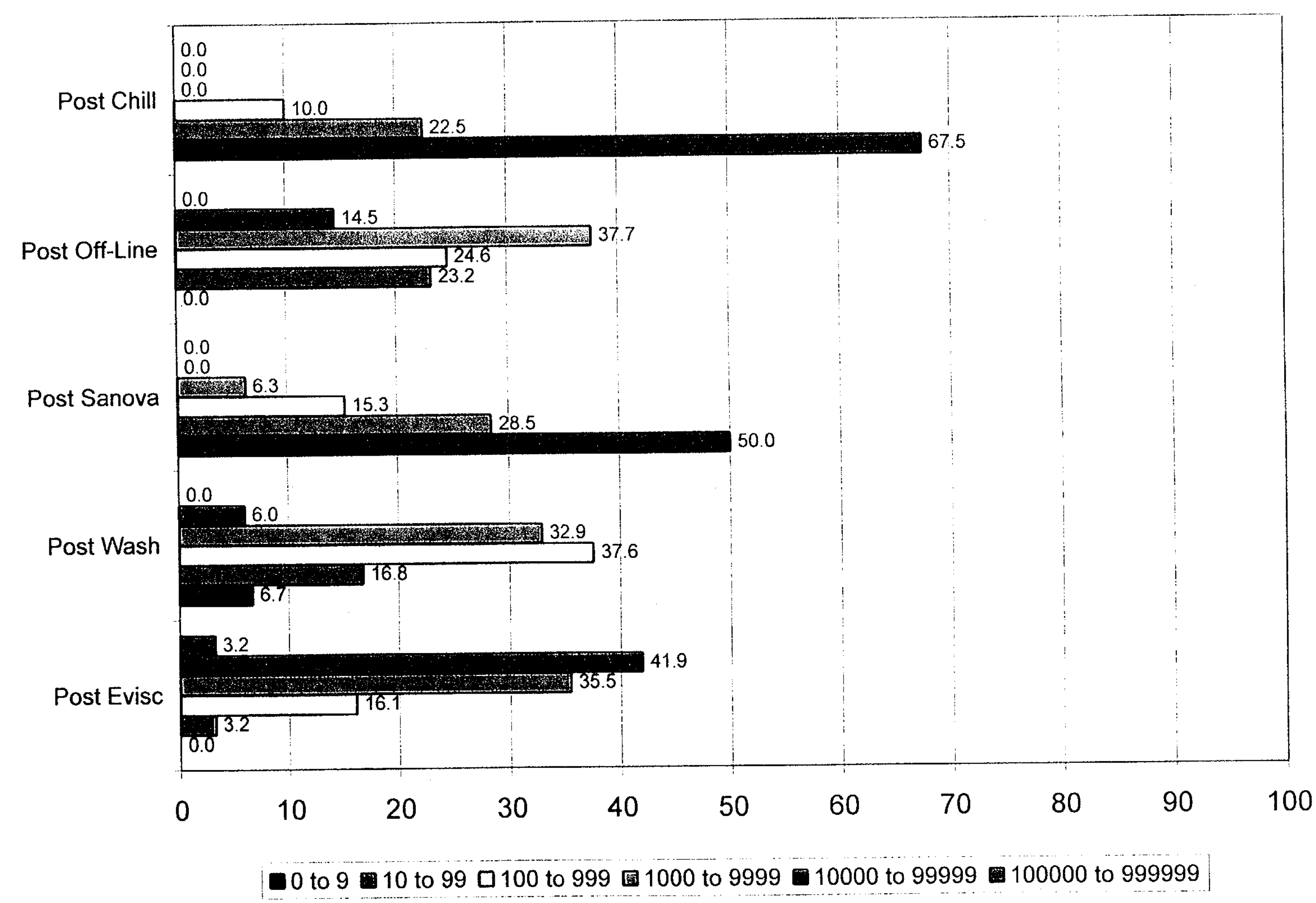
COP - All Data Combined
Campylobacter spp. Fresh Data Frequency Distribution

Data Range	Post Evisc	Post Wash	Post Sanova	Post Off-Line	Post Chill
n =	62	149	144	69	40
	%	%	%	%	%
0 to 9	0.0	6.7	50.0	0.0	67.5
10 to 99	3.2	16.8	28.5	23.2	22.5
100 to 999	16.1	37.6	15.3	24.6	10.0
1000 to 9999	35.5	32.9	6.3	37.7	0.0
10000 to 99999	41.9	6.0	0.0	14.5	0.0
100000 to 999999	3.2	0.0	0.0	0.0	0.0
Total	100	100	100	100	100

Campylobacter spp. - Fresh Data Frequency Distribution
All Studies Combined



000029

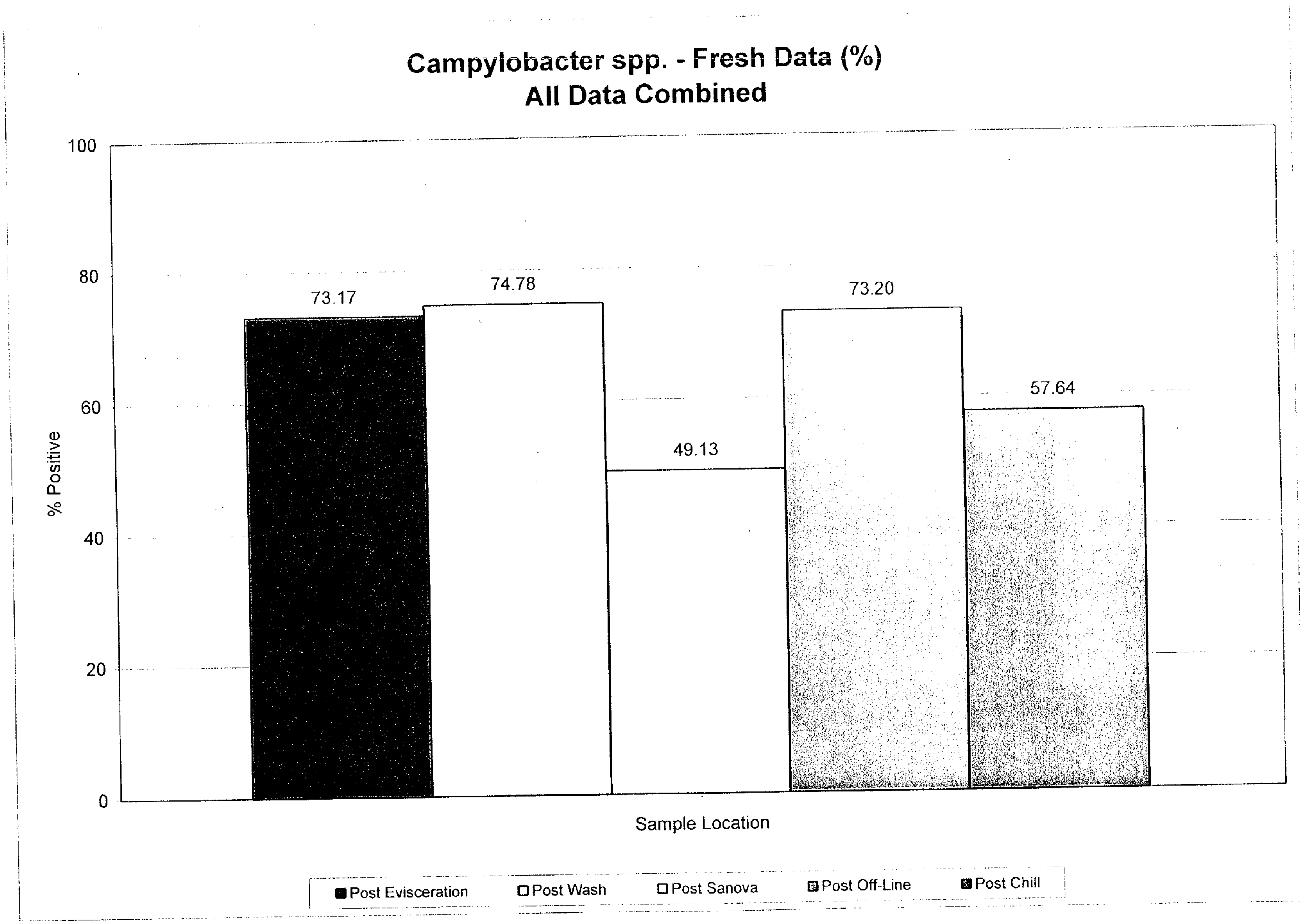
Incidence of Campylobacter spp. (%)
Summary Data

Experiment No.	n	Post Evisceration	n	Post Wash	n	Post Sanova	n	Post Off-line	n	Post Chill
020199PF - Phase II	70	100	70	100	70	100	70	100	63	100
031599GK - Phase II	36	85.00^{a}	30	56.67^{bc}	30	36.67^{c}	40	65.00^{b}	-	-
060199PF - Phase II	60	53.33^{bc}	50	86.00^{a}	50	38.00^{c}	60	65.00^{b}	60	48.33^{b}
072799TI - Phase II	40	52.50^{a}	40	40.00^{ab}	40	5.00^{c}	40	45.00^{ab}	40	27.50^{b}
072799TP - Phase II	40	62.50^{a}	40	65.00^{a}	40	35.00^{b}	40	75.00^{a}	40	35.00^{b}
Summary all data	246	73.17^{a}	230	74.78^{a}	230	49.13^{b}	250	73.20^{a}	203	57.64^{b}

(Within rows, means with no common superscript are significantly different, $p < 0.001$)

Average Campylobacter spp. **reduction** in incidence due to off-line reprocessing = 0%
Average Campylobacter spp. **reduction** in incidence due to Continuous On-line Processing = 24.04

000030


Campylobacter spp. - Fresh Data (%)
All Data Combined
100
80
60
40
20
0
% Positive
73.17
74.78
49.13
73.20
57.64
Sample Location
Post Evisceration
Post Wash
Post Sanova
Post Off-Line
Post Chill

000031

Comparison of Sample Handling Practices - Fresh versus Frozen Carcass Rinse Samples

E *coli.* – **Sample Distribution Post Wash**

Data Frequency Distribution		% Fresh	% Frozen
Unacceptable	> 1000	22.70	3.7
Marginal ("M")	100 to 999	47.6	24.1
Acceptable ("m")	0 to 100	29.8	72.2
Acceptable	< 10	1.1	26.1

Probability of Meeting USDA Compliance Criteria

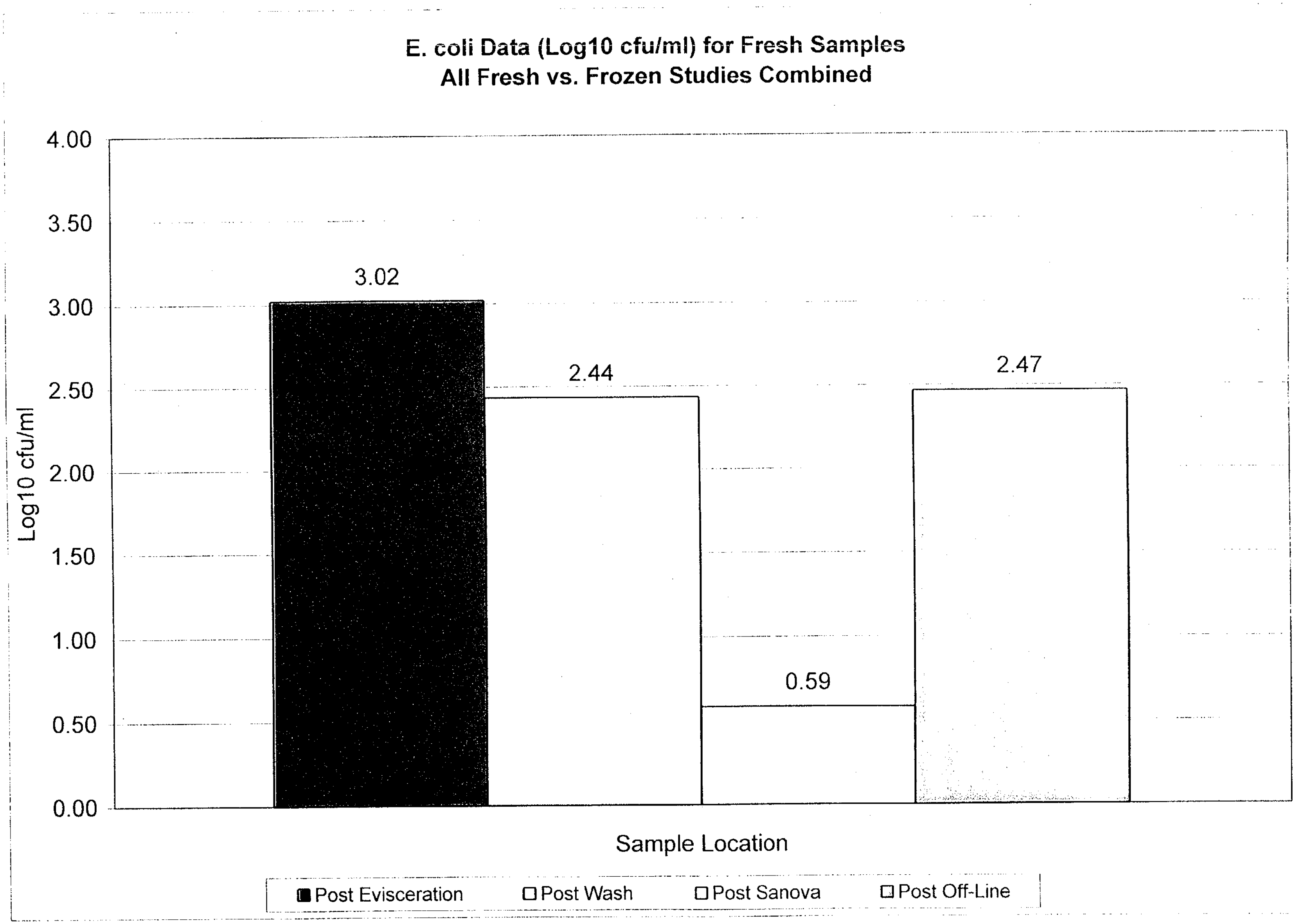
	Probability of Exceeding:	
	"m" = 100 cfu/ml	"M" = 1000 cfu/ml
Fresh	1:2	1:4
Frozen	1:4	1:27

On the assumption that pre-chill carcass *E. coli* counts and distribution frequencies do not change (worsen due to cross contamination) during chilling, "Frozen" carcass wash samples taken post-IOBW would have a significantly greater chance of passing current USDA "m" and "M" microbial limit criteria when compared with "Fresh" carcass wash samples taken at the same location.

Escherichia coli Counts ($\log_{10}$ cfu/ml) – Comparison of Fresh vs. Frozen Split Samples
Summary Data

Experiment No.	n	Post Evisceration	n	Post Wash	n	Post Sanova	n	Post Off-line
Fresh Samples								
072198PF - Phase I	-	-	30	2.74^{a}	30	0.99^{c}	30	2.51^{b}
020199PF - Phase II	80	3.29^{a}	79	2.93^{b}	80	1.11^{d}	80	2.37^{c}
021599GK - Phase I	120	2.82^{a}	120	2.04^{b}	120	0.48^{c}	120	2.20^{b}
050499PF - Phase I	120	3.04^{a}	120	2.43^{c}	120	0.25^{d}	120	2.79^{b}
Summary all fresh sample data	320	3.02^{ax}	349	2.44^{bx}	350	0.59^{cx}	350	2.47^{bx}
Frozen Samples								
072198PF - Phase I	-	-	30	1.33^{a}	30	0.56^{b}	30	1.36^{a}
020199PF - Phase II	80	2.22^{a}	80	1.98^{b}	78	0.19^{d}	80	1.35^{c}
021599GK - Phase I	118	2.03^{a}	120	1.05^{c}	120	0.06^{d}	120	1.35^{b}
050499PF - Phase I	120	2.37^{a}	119	1.72^{c}	119	0.01^{d}	120	1.93^{b}
Summary all frozen sample data	318	2.20^{ay}	347	1.51^{by}	347	0.11^{cy}	350	1.55^{by}

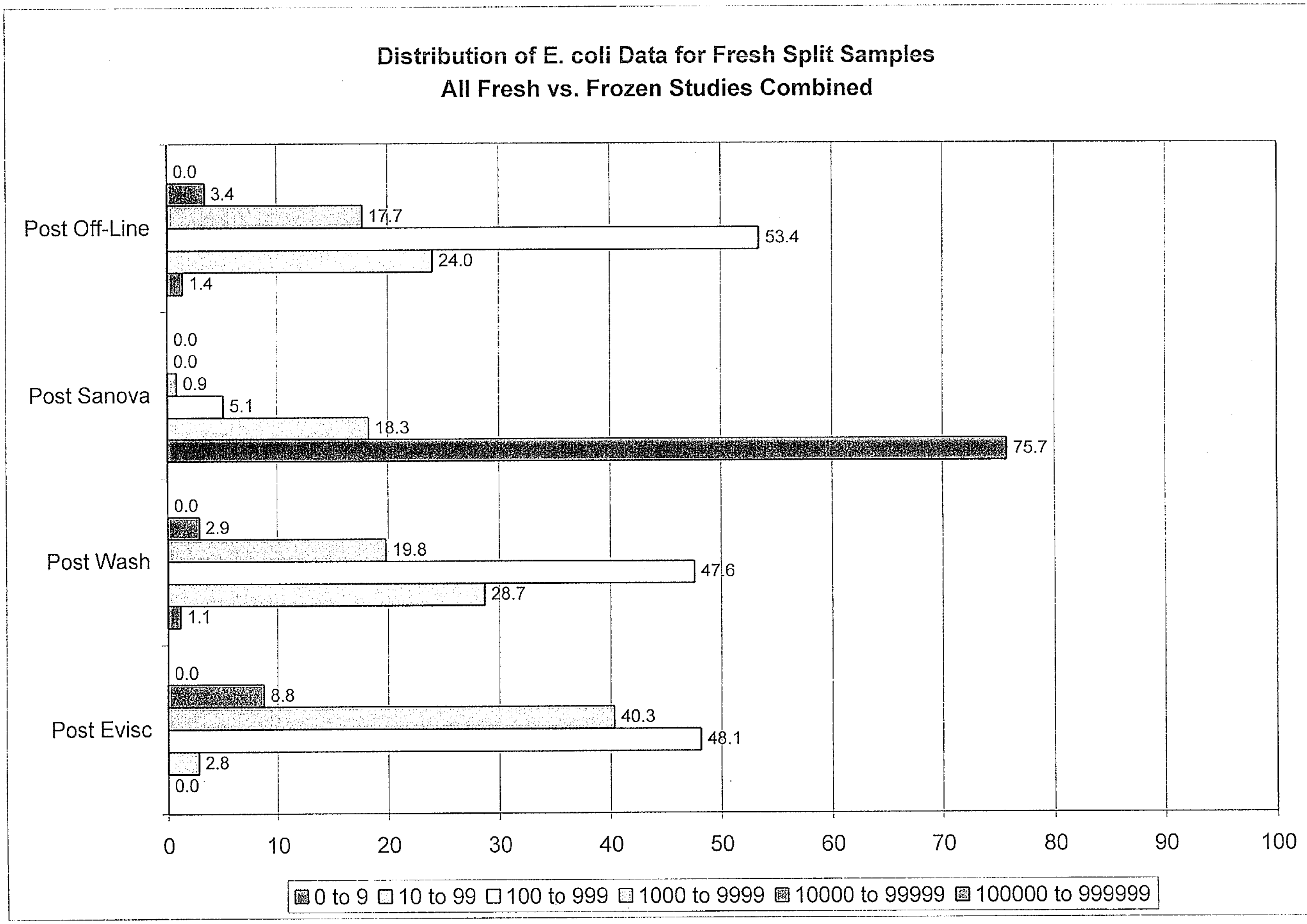
(Within rows, means with no common superscript (abcd) are significantly different, $p < 0.001$. For data in "summary" rows, numbers within column with no common superscript (xy) are significantly different, $p < 0.001$)


E. coli Data (Log10 cfu/ml) for Fresh Samples
All Fresh vs. Frozen Studies Combined
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Log10 cfu/ml
3.02
2.44
0.59
2.47
Sample Location
Post Evisceration
Post Wash
Post Sanova
Post Off-Line

000034

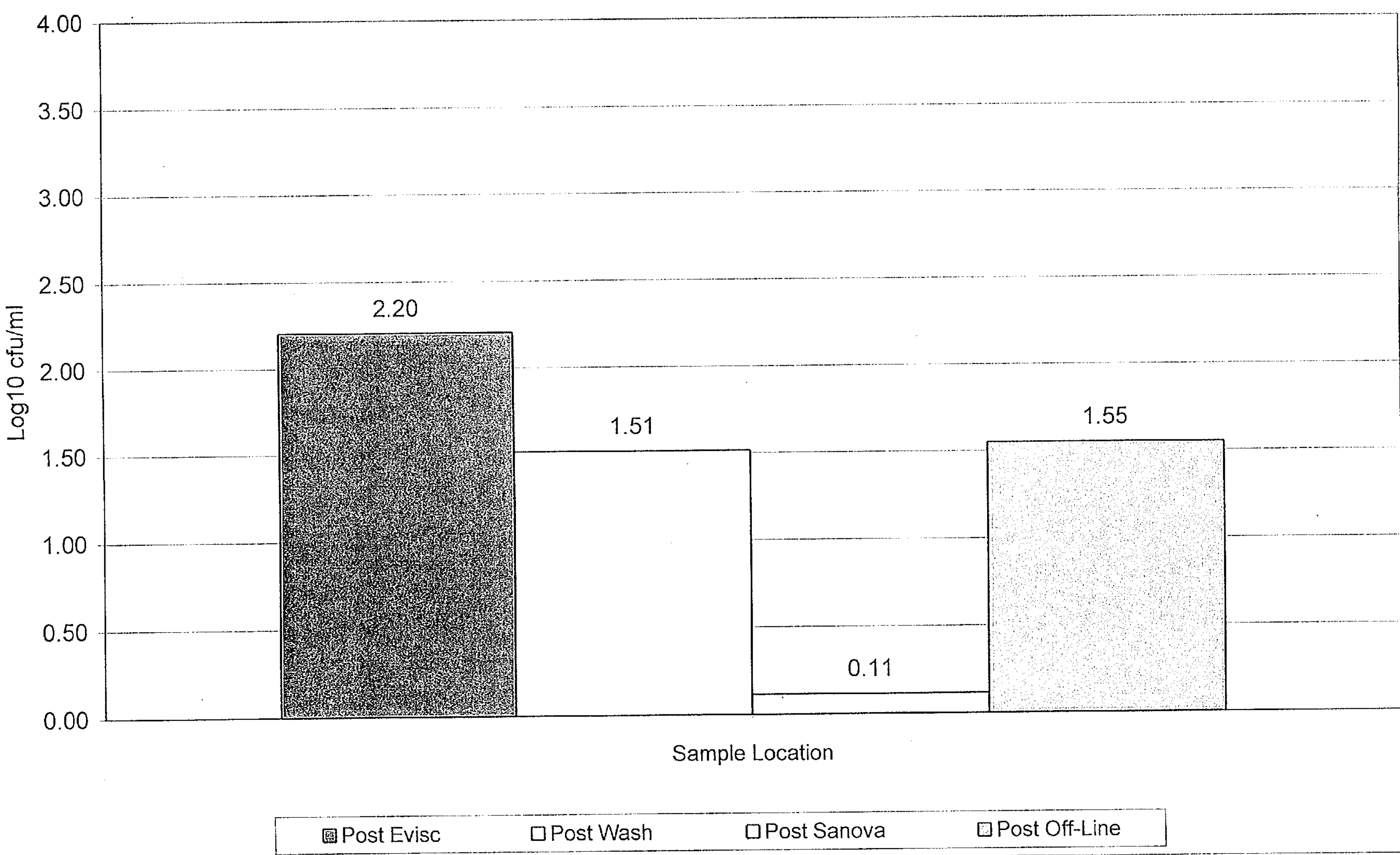
COP - All Fresh Split Samples Combined
***Escherichia coli* Data Frequency Distribution**

Data Range	Post Evisc	Post Wash	Post Sanova	Post Off-Line
n =	320	350	349	350
	%	%	%	%
0 to 9	0.0	1.1	75.7	1.4
10 to 99	2.8	28.7	18.3	24.0
100 to 999	48.1	47.6	5.1	53.4
1000 to 9999	40.3	19.8	0.9	17.7
10000 to 99999	8.8	2.9	0.0	3.4
100000 to 999999	0.0	0.0	0.0	0.0
Total	100.0	100.0	100.0	100.0


Distribution of E. coli Data for Fresh Split Samples
All Fresh vs. Frozen Studies Combined
Post Off-Line
0.0
3.4
17.7
53.4
24.0
1.4
Post Sanova
0.0
0.0
0.9
5.1
18.3
75.7
Post Wash
0.0
2.9
19.8
47.6
28.7
1.1
Post Evisc
0.0
8.8
40.3
48.1
2.8
0.0
0 10 20 30 40 50 60 70 80 90 100
0 to 9
10 to 99
100 to 999
1000 to 9999
10000 to 99999
100000 to 999999

000035

E. coli Data (Log10 cfu/ml) for Frozen Samples
All Fresh vs. Frozen Studies Combined

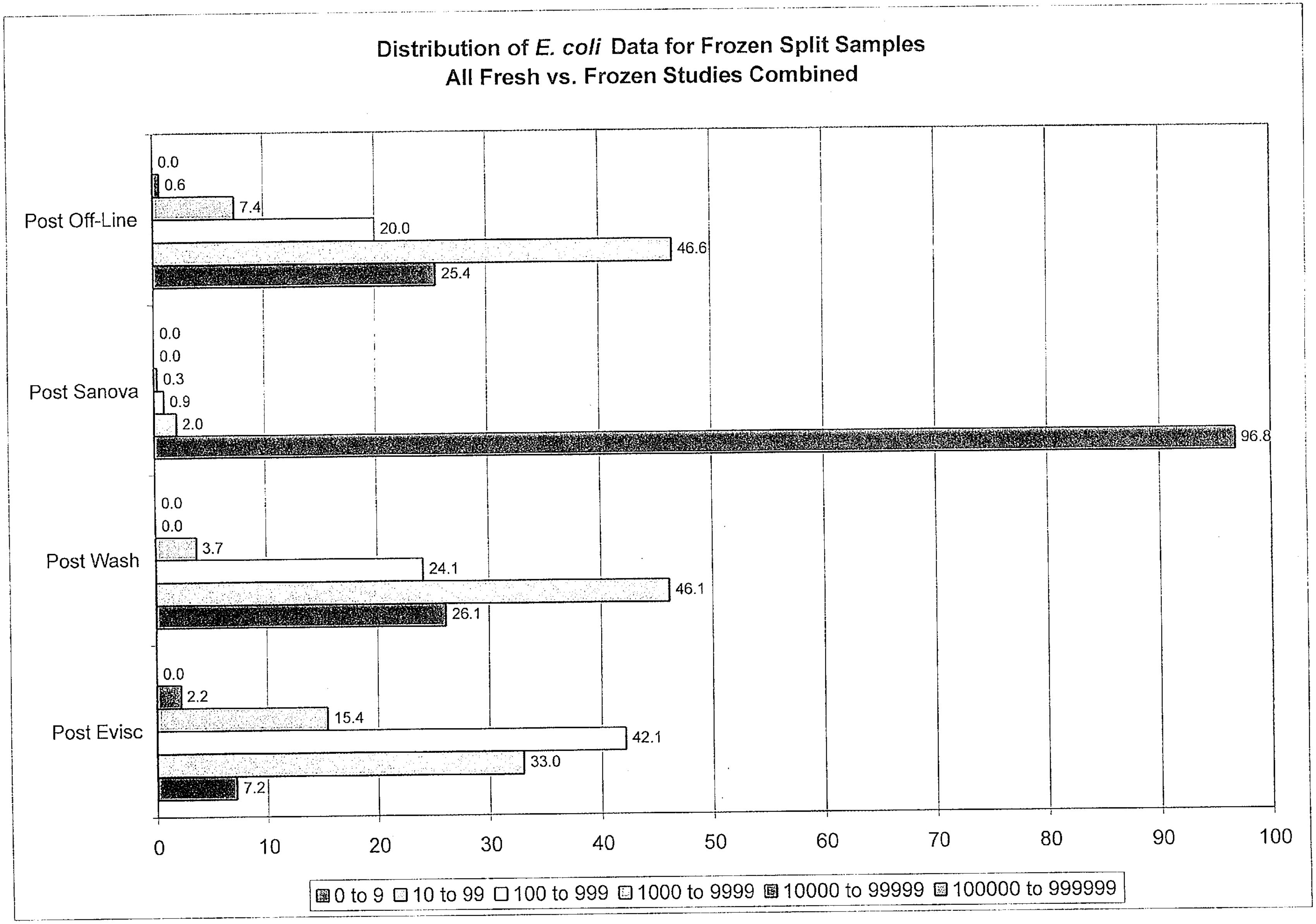


000037

COP - All Frozen Split Samples Combined
***Escherichia coli* Data Frequency Distribution**

Data Range	Post Evisc	Post Wash	Post Sanova	Post Off-Line
n =	318	350	349	347
	%	%	%	%
0 to 9	7.2	26.1	96.8	25.4
10 to 99	33.0	46.1	2.0	46.6
100 to 999	42.1	24.1	0.9	20.0
1000 to 9999	15.4	3.7	0.3	7.4
10000 to 99999	2.2	0.0	0.0	0.6
100000 to 999999	0.0	0.0	0.0	0.0
Total	100.0	100.0	100.0	100.0

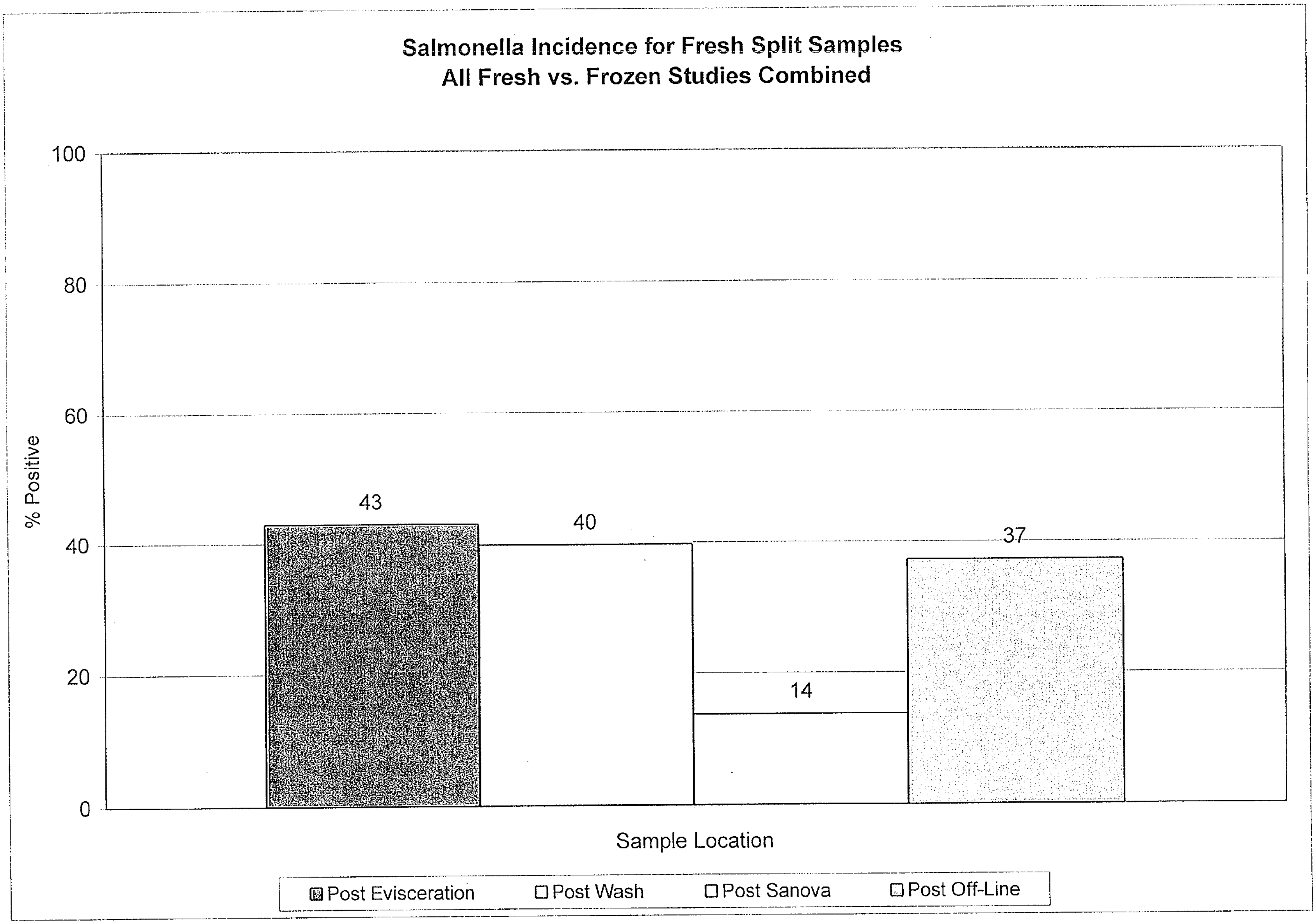
000038


Distribution of E. coli Data for Frozen Split Samples
All Fresh vs. Frozen Studies Combined
Post Off-Line
0.0
0.6
7.4
20.0
46.6
25.4
Post Sanova
0.0
0.0
0.3
0.9
2.0
96.8
Post Wash
0.0
0.0
3.7
24.1
46.1
26.1
Post Evisc
0.0
2.2
15.4
42.1
33.0
7.2
0
10
20
30
40
50
60
70
80
90
100
0 to 9
10 to 99
100 to 999
1000 to 9999
10000 to 99999
100000 to 999999

Incidence of Salmonella (%) – Comparison of Fresh vs. Frozen Split Samples
Summary Data

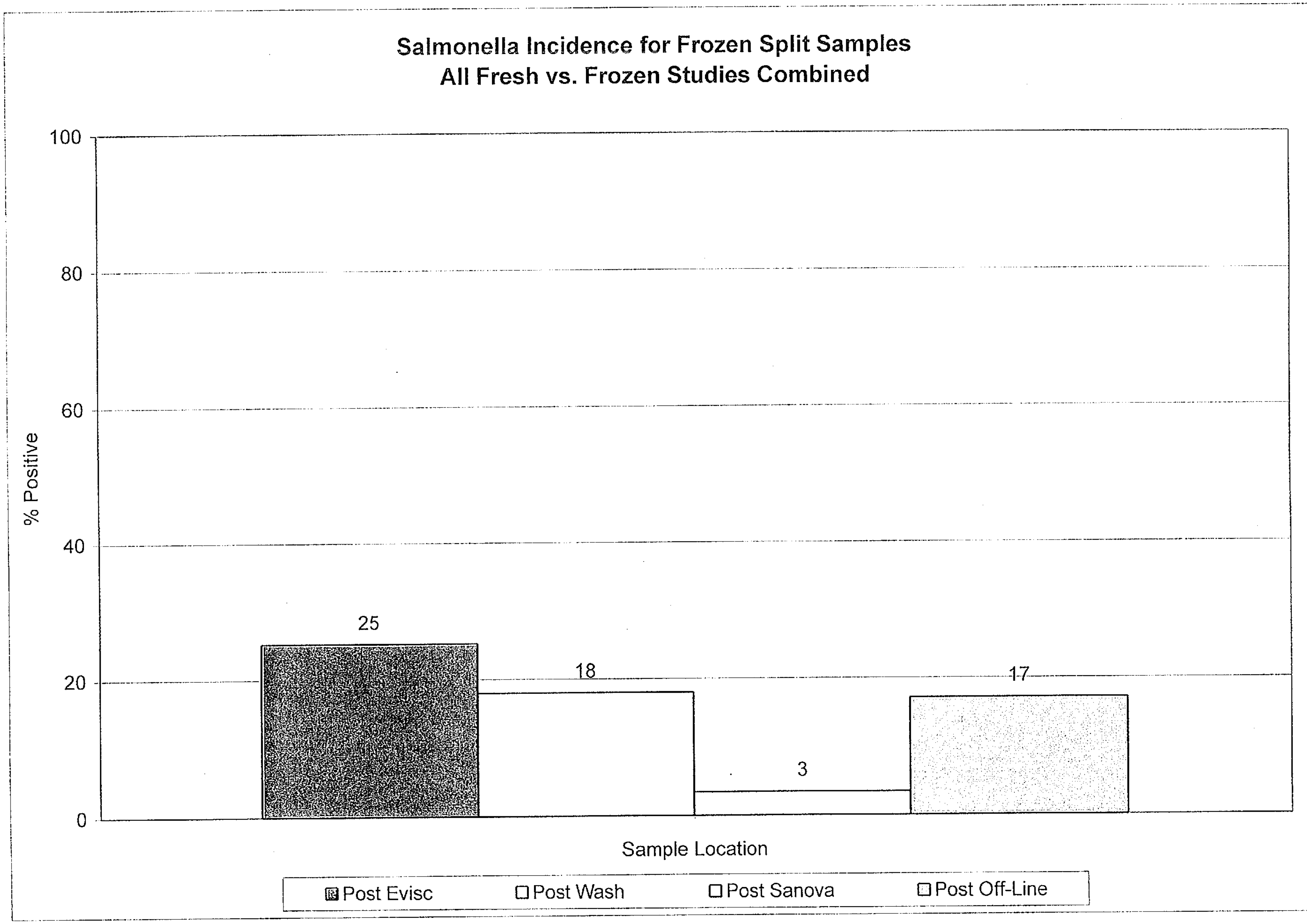
Experiment No.	n	Post Evisceration	n	Post Wash	n	Post Sanova	n	Post Off-line
Fresh Samples								
072198PF - Phase I	-	-	30	23.33^{b}	30	6.67^{c}	30	30.00^{a}
020199PF - Phase II	80	69.62^{a}	79	52.50^{b}	80	17.50^{c}	80	53.16^{b}
021599GK - Phase I	120	50.00^{a}	120	52.50^{a}	120	15.83^{c}	120	40.83^{b}
050499PF - Phase I	120	18.33^{b}	120	22.50^{a}	120	10.92^{c}	120	25.00^{a}
Summary all fresh sample data	320	42.95^{ax}	349	39.71^{bx}	350	13.75^{cx}	350	37.25^{bx}
Frozen Samples								
072198PF - Phase I	-	-	30	10.00^{a}	30	3.33^{b}	30	3.33^{b}
020199PF - Phase II	80	32.50^{a}	80	23.75^{b}	78	7.50^{d}	80	16.25^{c}
021599GK - Phase I	118	37.29^{a}	120	20.83^{c}	120	2.52^{d}	120	25.83^{b}
050499PF - Phase I	120	8.40^{c}	119	13.33^{a}	119	1.67^{d}	120	12.50^{b}
Summary all frozen sample data	318	25.24^{ay}	347	18.00^{by}	347	3.44^{cy}	350	17.14^{by}

(Within rows, means with no common superscript (abcd) are significantly different, $p < 0.001$. For data in "summary" rows, numbers within column with no common superscript (xy) are significantly different, $p < 0.001$)



Salmonella Incidence for Fresh Split Samples
All Fresh vs. Frozen Studies Combined
100
80
60
40
20
0
% Positive
43
40
14
37
Sample Location
Post Evisceration
Post Wash
Post Sanova
Post Off-Line

000041


Salmonella Incidence for Frozen Split Samples
All Fresh vs. Frozen Studies Combined
100
80
60
40
20
0
% Positive
25
18
3
17
Sample Location
Post Evisc
Post Wash
Post Sanova
Post Off-Line

000042

PROJECT FINAL REPORT
MICROBIOLOGY DEPARTMENT
ABC RESEARCH CORPORATION

DATE: June 17, 1999

PREPARED FOR: Alcide Corporation

CLIENT CONTACT: Dr. G. Kere Kemp, Vice President

PROJECT: Protocol for Validation of Thiosulfate Used as a Neutralizer for ASC Chemistry in Microbiological Sample Materials.

OBJECTIVE:

This study examined the use of thiosulfate in poultry rinse buffer. This study was separated into four phases. The original proposal consisted of three phases, a fourth was added to confirm the efficacy of ASC. The study was broken down as follows:

- Effects of thiosulfate concentration on microbial survival.
- Microbiological effect of 0.1% thiosulfate on ASC inactivation.
- Effect of ASC concentration on microbial survival in the absence of thiosulfate.
- Chemical effect of 0.1% thiosulfate on ASC inactivation.

CHEMISTRY

Alcide Corporation provided the chemicals used in this experiment. The chemistry was pre-measured by Alcide Corporation to deliver 1200 ppm when mixed according to manufacturer's specification. The lot numbers used are as follows:

	Savona Activator	Sanova Base
Phase 1 (microbiology)	AC071-070A	AC071-070B
Phase 2 (microbiology)	AC071-070A	AC071-070B
Phase 3 (microbiology)	AC070-116A	AC070-116B
Phase 4 (chemistry)	AC070-116A	AC070-116B

PROCEDURES:

This study followed the procedures outlined in an e-mailed file from Dr. Kere Kemp, dated 5/6/99, with the exception of the portion that examined the effect of ASC concentration on microbial survival in the absence of thiosulfate. This protocol is presented below.

Phase 1: Effects of Thiosulfate Concentration on Microbial Survival.

Use the test tube dilution test methodology
Cultivate E. coli with titers in the range 10^4 to 10^5
5 replicates per concentration of thiosulfate to be tested
Inoculate 1 ml volumes of E. coli into 9 ml volumes of the thiosulfate solution at concentrations of 0.0%, 0.01%, 0.1%, 1.0% and 5.0%
Plate and count utilizing E. coli/coliform Petrifilm™

Phase 2: Effect of 0.1% Thiosulfate on ASC inactivation.

400 ml volumes (or proportions thereof) of BPD containing 0.1% thiosulfate
Inoculate with an appropriate volume of E. coli (titer in the range 10^4 to 10^5)
Add freshly mixed ASC (citric acid activated, pH 2.5, 1200 ppm) in volumes of 50ml, 25 ml, 10ml, 5ml, 2.5ml, 0ml
Wait 5 minutes then plate and count utilizing E. coli/coliform Petrifilm™
5 replicates per volume of ASC
Repeat using Butterfield's diluent

Phase 3: Effect of ASC concentration on microbial inactivation.

400 ml volumes (or proportions thereof) of BPD containing no thiosulfate
Inoculate with an appropriate volume of E. coli (titer in the range 10^4 to 10^5)
Add freshly mixed ASC (citric acid activated, pH 2.5, 1200 ppm) in volumes of 25 ml, 10ml, 5ml, 2.5ml, 0ml
Wait 5 minutes then plate and count utilizing E. coli/coliform Petrifilm™
5 replicates per volume of ASC
Repeat using Butterfield's diluent

Phase 4: Effect of 0.1% Thiosulfate on ASC inactivation.

400 ml volumes (or proportions thereof) of BPD containing 0.1% thiosulfate
Add freshly mixed ASC (citric acid activated, pH 2.5, 1200 ppm) in volumes of 50ml, 25 ml, 10ml, 5ml, 2.5ml, 0ml
Wait 5 minutes then assay for residual chlorite by iodometric titration plus check pH.
5 replicates per volume of ASC
Repeat using Butterfield's diluent

RESULTS

The results of this study can be seen in the following Tables: (1) Effects of Thiosulfate Concentration on Microbial Survival; (2) Effect of 0.1% Thiosulfate on ASC inactivation; (3) Effect of ASC concentration on microbial inactivation; and (4) Effect of 0.1% Thiosulfate on ASC Inactivation. Tests for phases 1 and 2 were performed five minutes after inoculation (day 0) and after 24 hours of refrigerated storage at 38-40°F (day 1). No results are given for chlorite concentration in Table 4 (listed as n/a) due to complete inactivation of the chemistry.

The results seen in Table 1 show virtually no reduction associated with increases in thiosulfate concentration in Butterfield's Phosphate Buffer (BPB). It should be noted that for day 0 and a thiosulfate concentration of 0%, the highest recovered value was recorded. Although it is difficult to ascertain if this approximate 0.5 log reduction as compared with other sample points is significant, all other points examined do not appear to be effected by either thiosulfate concentration or cold storage.

The next phase examined the ability of 0.1% thiosulfate to inactivate various concentrations of ASC in both BPB and Buffered Peptone Water (BPW). As seen in Table 2, no reductions in bacterial numbers were observed for any of the concentrations of ASC applied each of the test buffers. Little difference was seen in recovery from day 0 as compared to day 1. The differences seen for BPW were less than were observed for BPB, although the initial counts for BPB were slightly higher.

The effect of ASC on buffers containing no thiosulfate can be seen in Table 3. As expected, as the concentration of ASC increased in BPB, the levels of recovered E. coli decreases. Significant reduction was observed at the 5.0 ml and high dose levels. The results observed for BPW were significantly different from those seen for BPB. Despite the lack of thiosulfate and ASC concentrations as high as 25 ml/per 400 ml, no reduction was observed in BPW.

The final phase of this study examined the chemical effects various concentrations of ASC added to both BPB and BPW (both with 0.1% thiosulfate). As seen in Table 4, the 0.1% thiosulfate completely inactivated the all doses of ASC applied. The BPB experienced greater deflections in pH as ASC concentrations increased compared with BPW. Even with the addition of 50 ml of ASC, the pH was within one log of the initial value. At the same ASC concentration, the pH for the BPB decreased by three log units.

DISCUSSION

The results of this study showed that the inclusion of 0.1% thiosulfate was adequate to neutralize up to 50 ml of ASC (1200 ppm). Both buffers appeared to have similar recovery characteristics. The effects seen in Table 3 display some interesting results. It is clear that BPB without thiosulfate is inadequate to use as a poultry rinse when excess amounts of chlorine are captured in the buffer solution. Conversely, the BPW was effective in maintaining bacterial counts, even in the presence of 50 ml of ASC. One explanation could be the lack of effectiveness of ASC at higher pH levels. Therefore, by maintaining a high pH, the efficacy of ASC diminishes. This effect would then not occur when using other halogen chemistry that remain active in the pH 6 to 7 range (i.e., $HOCl/OCl^-$).

To ascertain if the small variations seen between day 0 samples with 0% and 0.1% thiosulfate were significant, a larger group of sample could be taken. Therefore, based on the information generated in this limited-scope study, either a BPB or BPW buffer with 0.1% thiosulfate would provide an added margin of safety when dealing with halogen disinfectants then either diluent without a neutralizing agent .

Table 1: Effects of Thiosulfate Concentration on Microbial Survival				
	Day 0		Day 1	
Thiosulfate Conc.	Rep	CFU/ml	Rep	CFU/ml
0%				
	A	3.70E+05	A	1.90E+04
	B	3.00E+04	B	2.40E+04
	C	1.00E+06	C	8.00E+04
	D	8.00E+04	D	1.16E+05
	E	1.48E+06	E	7.60E+04
Mean		**5.92E+05**		**1.22E+05**
0.01%	A	1.28E+05	A	7.20E+04
	B	1.20E+05	B	1.64E+05
	C	1.24E+05	C	1.12E+05
	D	1.40E+05	D	1.72E+05
	E	7.20E+04	E	9.20E+04
Mean		**1.17E+05**		**9.76E+04**
0.10%	A	9.60E+04	A	8.40E+04
	B	1.52E+05	B	1.24E+05
	C	9.60E+04	C	9.20E+04
	D	6.80E+04	D	1.00E+05
	E	6.40E+04	E	8.80E+04
Mean		**9.52E+04**		**9.76E+04**
1.00%	A	1.00E+05	A	7.00E+04
	B	7.20E+04	B	6.60E+04
	C	1.00E+05	C	7.80E+04
	D	1.02E+05	D	6.00E+04
	E	8.80E+04	E	9.60E+04
Mean		**9.24E+04**		**7.40E+04**
5.00%	A	1.32E+05	A	6.40E+04
	B	1.00E+05	B	5.60E+04
	C	6.00E+04	C	1.04E+05
	D	8.80E+04	D	5.40E+04
	E	1.00E+05	E	6.80E+04
Mean		**9.60E+04**		**6.92E+04**

Table 2: Effect of 0.1% Thiosulfate on ASC inactivation									
BPW					BPB				
	Day 0		Day 1			Day 0		Day 1	
ASC Vol. (ml)	Rep	CFU/ml	Rep	CFU/ml	ASC Vol. (ml)	Rep	CFU/ml	Rep	CFU/ml
0.0					0.0				
	A	5.00E+05	A	9.60E+04		A	9.00E+04	A	1.08E+05
	B	1.40E+05	B	1.38E+05		B	1.12E+05	B	8.80E+04
	C	1.00E+05	C	4.00E+04		C	1.14E+05	C	8.00E+04
	D	1.28E+05	D	5.00E+04		D	8.00E+04	D	8.40E+04
	E	8.40E+04	E	5.00E+05		E	1.36E+05	E	1.08E+02
Mean		**1.90E+05**		**1.65E+05**	**Mean**		**1.06E+05**		**7.20E+04**
2.5	A	1.00E+05	A	6.40E+04	2.5	A	1.20E+05	A	8.40E+04
	B	6.00E+04	B	6.60E+04		B	**1.50E+05**	B	1.16E+05
	C	7.60E+04	C	8.40E+04		C	1.24E+05	C	6.00E+04
	D	8.00E+04	D	7.40E+04		D	1.48E+05	D	7.20E+04
	E	6.40E+04	E	8.60E+04		E	1.00E+05	E	5.80E+04
Mean		**7.60E+04**		**7.48E+04**	**Mean**		**1.28E+05**		**7.80E+04**
5.0	A	1.00E+05	A	9.00E+04	5.0	A	8.40E+04	A	8.80E+04
	B	8.80E+04	B	8.20E+04		B	1.00E+05	B	5.40E+04
	C	8.40E+04	C	7.60E+04		C	1.40E+05	C	7.20E+04
	D	9.20E+04	D	7.20E+04		D	1.16E+05	D	8.80E+04
	E	5.40E+04	E	5.40E+04		E	**1.50E+05**	E	5.40E+04
Mean		**8.36E+04**		**7.48E+04**	**Mean**		**1.18E+05**		**7.12E+04**
10.0	A	9.20E+04	A	6.60E+04	10.0	A	1.32E+05	A	4.80E+04
	B	1.28E+05	B	6.60E+04		B	**1.50E+05**	B	5.40E+04
	C	1.16E+05	C	9.20E+04		C	1.44E+05	C	3.80E+04
	D	1.40E+05	D	**1.50E+05**		D	**1.36E+05**	D	**5.40E+04**
	E	1.20E+05	E	9.20E+04		E	6.80E+04	E	3.80E+04
Mean		**1.19E+05**		**9.32E+04**	**Mean**		**1.26E+05**		**4.64E+04**
25.0	A	1.02E+05	A	5.00E+04	25.0	A	1.08E+05	A	5.40E+04
	B	7.60E+04	B	5.00E+04		B	1.48E+05	B	6.20E+04
	C	7.20E+04	C	4.80E+04		C	1.44E+05	C	7.40E+04
	D	1.02E+05	D	6.20E+04		D	1.08E+05	D	5.60E+04
	E	9.20E+04	E	1.28E+05		E	**1.50E+05**	E	3.20E+04
Mean		**8.88E+04**		**6.76E+04**	**Mean**		**1.32E+05**		**5.56E+04**
50.0	A	1.12E+05	A	4.60E+04	50.0	A	1.12E+05	A	4.80E+04
	B	9.20E+04	B	4.40E+04		B	1.16E+05	B	8.40E+04
	C	6.80E+04	C	6.20E+04		C	1.04E+05	C	8.00E+04
	D	1.28E+05	D	5.20E+04		D	**1.50E+05**	D	6.20E+04
	E	9.20E+04	E	3.00E+04		E	1.32E+05	E	7.20E+04
Mean		**9.84E+04**		**4.68E+04**	**Mean**		**1.23E+05**		**6.92E+04**

Table 3: Effect of ASC concentration on microbial inactivation					
BPB			BPW		
ASC Vol. (ml)	Rep	CFU/ml	ASC Vol. (ml)	Rep	CFU/ml
0.0			0.0		
	A	2.70E+04		A	4.30E+04
	B	1.70E+04		B	5.50E+04
	C	4.00E+04		C	3.40E+04
	D	3.80E+04		D	4.40E+04
	E	4.20E+04		E	4.10E+04
Mean		**3.28E+04**	**Mean**		**4.34E+04**
2.5	A	3.40E+04	2.5	A	5.60E+04
	B	2.70E+04		B	**1.50E+05**
	C	2.80E+04		C	4.10E+04
	D	2.70E+04		D	3.40E+04
	E	2.30E+04		E	3.20E+04
Mean		**2.78E+04**	**Mean**		**6.26E+04**
5.0	A	3.50E+02	5.0	A	3.90E+04
	B	*8.00E+01*		B	3.90E+04
	C	*3.00E+01*		C	3.90E+04
	D	*3.00E+01*		D	4.00E+04
	E	*2.00E+01*		E	4.10E+04
Mean		**1.02E+02**	**Mean**		**3.96E+04**
10.0	A	**1.00E+01**	10.0	A	4.30E+04
	B	**1.00E+01**		B	4.10E+04
	C	**1.00E+01**		C	4.20E+04
	D	*1.00E+01*		D	4.40E+04
	E	*1.00E+01*		E	5.20E+04
Mean		**1.00E+01**	**Mean**		**4.44E+04**
25.0	A	*1.00E+01*	25.0	A	3.30E+04
	B	*1.00E+01*		B	3.20E+04
	C	*1.00E+01*		C	3.90E+04
	D	*1.00E+01*		D	4.50E+04
	E	*1.00E+01*		E	2.70E+04
Mean		**1.00E+01**	**Mean**		**3.52E+04**

Table 4: Effect of 0.1% Thiosulfate on ASC Inactivation									
	BPB					BPW			
ASC Vol. (ml)	Rep	**Chlorite**	Rep	**pH**	ASC Vol. (ml)	Rep	**Chlorite**	Rep	**pH**
0.00					0.00				
	A	n/a	A	7.10		A	n/a	A	7.36
	B	n/a	B	7.02		B	n/a	B	7.26
	C	n/a	C	7.04		C	n/a	C	7.33
	D	n/a	D	7.00		D	n/a	D	7.35
	E	n/a	E	7.05		E	n/a	E	7.35
Mean		**n/a**		**7.04**	**Mean**		**n/a**		**7.33**
2.5	A	n/a	A	5.14	2.5	A	n/a	A	7.32
	B	n/a	B	5.05		B	n/a	B	7.21
	C	n/a	C	4.95		C	n/a	C	7.31
	D	n/a	D	4.96		D	n/a	D	7.32
	E	n/a	E	4.97		E	n/a	E	7.31
Mean		**n/a**		**5.01**	**Mean**		**n/a**		**7.29**
5.0	A	n/a	A	4.72	5.0	A	n/a	A	7.27
	B	n/a	B	4.75		B	n/a	B	7.16
	C	n/a	C	4.65		C	n/a	C	7.27
	D	n/a	D	4.56		D	n/a	D	7.27
	E	n/a	E	4.57		E	n/a	E	7.27
Mean		**n/a**		**4.65**	**Mean**		**n/a**		**7.25**
10.0	A	n/a	A	4.27	10.0	A	n/a	A	7.18
	B	n/a	B	4.52		B	n/a	B	7.07
	C	n/a	C	4.47		C	n/a	C	7.18
	D	n/a	D	4.41		D	n/a	D	7.18
	E	n/a	E	4.36		E	n/a	E	7.18
Mean		**n/a**		**4.41**	**Mean**		**n/a**		**7.16**
25.0	A	n/a	A	4.11	25.0	A	n/a	A	6.95
	B	n/a	B	4.21		B	n/a	B	6.83
	C	n/a	C	4.35		C	n/a	C	6.96
	D	n/a	D	4.21		D	n/a	D	6.95
	E	n/a	E	4.16		E	n/a	E	6.95
Mean		**n/a**		**4.21**	**Mean**		**n/a**		**6.93**
50.0	A	n/a	A	3.98	50.0	A	n/a	A	6.60
	B	n/a	B	3.98		B	n/a	B	6.44
	C	n/a	C	4.06		C	n/a	C	6.66
	D	n/a	D	4.20		D	n/a	D	6.46
	E	n/a	E	4.03		E	n/a	E	6.61
Mean		**n/a**		**4.05**	**Mean**		**n/a**		**6.55**