International Fishmeal & Oil Manufacturers Association

RING TRIAL OF SALMONELLA AND ENTEROBACTERIACEAE ANALYSIS

Summary of Results for Laboratories

by

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RING TRIAL OF SALMONELLA AND ENTEROBACTERIACEAE ANALYSIS

Executive Summary

Five fish meal samples were prepared by the organising laboratory. The samples included a blank control, two samples contaminated with a low level of Salmonella and two samples contaminated with a high level.

The samples were sent to 14 participating laboratories (see Appendix 1) which were invited to use their own analytical techniques for identifying which samples were contaminated with Salmonella, the level of contamination and the specific sero group of Salmonella. In addition, the laboratories were invited to report the total enterobacteriaceae count.

All laboratories participating in this ring trial successfully isolated Salmonella from the two samples heavily contaminated with Salmonella. One laboratory failed to isolate Salmonella from both samples with a low level of contamination and two laboratories failed to isolate Salmonella from one of the low level contamination samples.

One laboratory isolated Salmonella from the Salmonella-negative control sample.

Seven laboratories sero-grouped their Salmonella isolates but only four reported the intended sero types.

Five laboratories reported enterobacteriaceae counts which were well outside the expected range.

This ring trial has lead to improvements in analytical procedures by some of the participating laboratories based on discussions with the organising laboratory.

It is proposed that this type of ring test become routine within Association Member laboratories leading to the possibility of an IFOMA seal of approval to those laboratories routinely capable of undertaking satisfactory analytical procedures.

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1. GENERAL INFORMATION

Distribution

Five samples were sent to 14 laboratories.

Types of Sample

Five fish meal samples were despatched. The samples included a blank control sample (0203), two samples (0202 and 0205) contaminated with a low level (approx. 5 cfu/g) of *Salmonella typhimurium* and two samples (0201 and 0204) contaminated with a high level (approx. 50,000 cfu/g) of either *Salmonella typhimurium* or *Salmonella tennessee* respectively.

Requests

Laboratories were requested to examine all samples for *Salmonella* and assess Enterobacteriaceae counts in samples 0201, 0203 and 0204.

Quality Control

Samples were despatched to participating laboratories, including the Quality Control Department. Ten lots of each sample were tested during the trial period by the Quality Control Department and provided the intended results.

Intended Results

Table 1 gives an overview of intended results for *Salmonella* isolation and Enterobacteriaceae counts and are derived from the analyses of multiple samples in the Quality Control Department.

Table 1: Overview of the intended results

Sample No.	Salmonella	Serogroup	Enterobacteriaceae Count (cfu/g)
08/0201	*S.typhimurium*	B	4.8×10^4
08/0202	*S.typhimurium*	B	<10
08/0203	Negative		<10
08/0204	*S.tennessee*	C_1-C_4	6.8×10^4
08/0205	*S.typhimurium*	B	<10

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2. SUMMARY OF PARTICIPANTS' RESPONSE AND RESULTS

Participation

All 14 participating laboratories analysed the received samples and returned a report.

Overall Results

Table 2: Summary of the results obtained by all participants

Sample No.	*Salmonella* Isolations[1]	Correctly reported Enterobacteriaceae Counts[2]
08/0201	14/14	8/14
08/0202	11/14	
08/0203	1/14	14[a]/14
08/0204	14/14	9/14
08/0205	13/14	

1 No. of participants isolating *Salmonella* / Total No. participants reporting

2 No. of participants reporting Enterobacteriaceae counts within 5th-95th percentile range of intended counts (Figures 1 and 2) /Total No. of participants reporting

a Counts reported as not detected, 0 or no count were interpreted as meaning less then 10 cfu/g

Figures 1 and 2 summarise the frequency distribution of Enterobacteriaceae counts produced by participants and the Quality Control Department (samples 0201 and 0204).

Figure 1: Enterobacteriaceae counts reported by participant ☐ and the reference range detected by the Quality Control Department ■ for sample 0201.

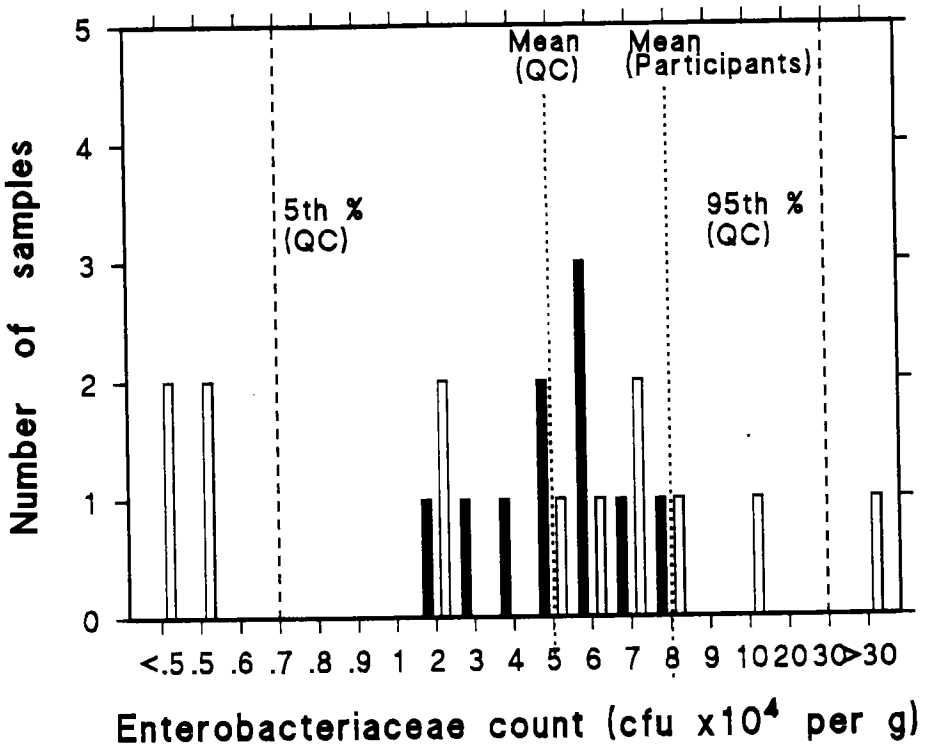

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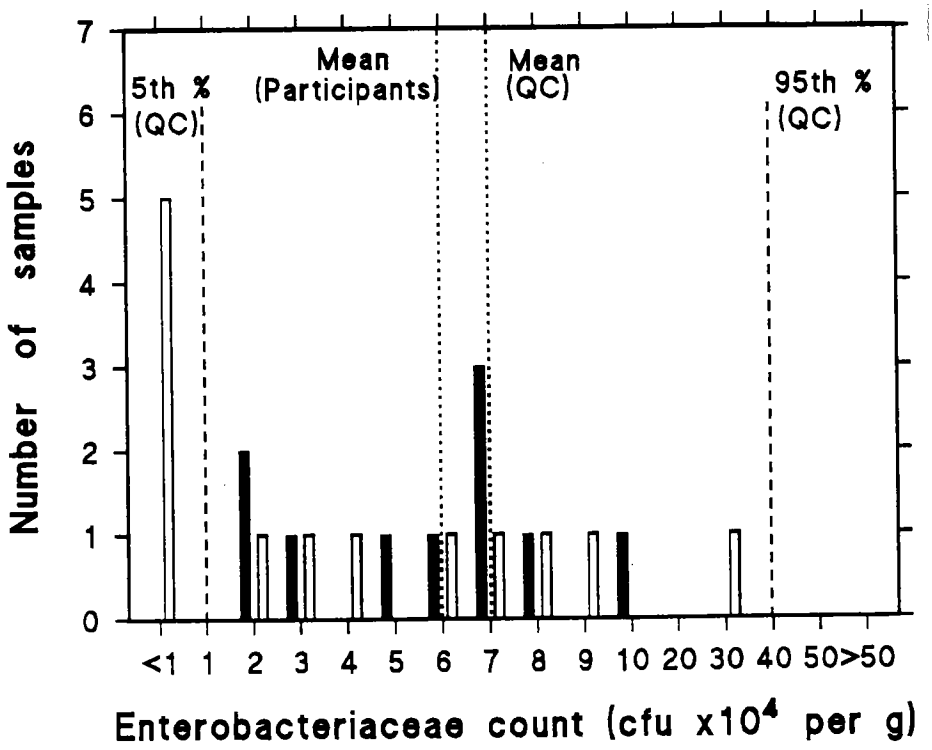
Figure 2: Enterobacteriaceae counts reported by participant □ and the reference range detected by the Quality Control Department ■ for sample 0204.



Tables 3-6 show the reported results for each participating laboratory

Table 3: Participants' results and intended result for sample 0201

Lab No.	*Salmonella*		Enterobacteriaceae cfu/g
	Isolated	Serogroup	
Intended	+	B	**4.8×10^4**
010	+	B	4.8×10^3
011	+	B	$>1.1 \times 10^4$
012	+	NR	4.2×10^5
013	+	NR	1.1×10^5
014	+	NR	7.0×10^4
015	+	NR	7.2×10^4
016	+	NR	8.4×10^4
017	+	NR	2.4×10^4
018	+	NR	6.3×10^4
019	+	B	2.4×10^2
020	+	A	1.9×10^4
021	+	C_1	4.9×10^3
022	+	NR	3.6×10^3
023	+	B	5.1×10^4
Recovery Rate	14/14		Mean: 7.8×10^4

+ Isolated NR Not Reported

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Table 4: Participants' results and intended result for samples 0202 and 0205.

Lab No.	*Salmonella* Sample 0202		*Salmonella* Sample 0205		Enterobacteriaceae cfu/g Sample 0202	Enterobacteriaceae cfu/g Sample 0205
	Isolated	Serogroup	Isolated	Serogroup		
Intended	**+**	**B**	**+**	**B**	**<10**	**<10**
010	+	B	+	C_1	5	200
011	+	B	+	B	<0.3	9.3
012	+	NR	+	NR	0	20
013	+	NR	+	NR	ND	ND
014	+	B	+	B	ND	ND
015	+	NR	+	NR	<10	<10
016	+	NR	+	NR	ND	ND
017	-		+	NR	ND	ND
018	+	NR	+	NR	<10	5
019	-		-		<20	<20
020	+	A	+	A	<10	10
021	+	C_1	+	C_1	0	0
022	-		+	NR	ND	ND
023	+	B	+	B	ND	ND
Recovery Rate	11/14		13/14		ND	ND

\+ Isolated
\- Not Isolated
NR Not Reported

ND Not Detected

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Table 5: Participants' results and intended result for sample 0203

Lab No.	*Salmonella*		Enterobacteriaceae cfu/g
	Isolated	Serogroup	
Intended	**-**		**<10**
010	-		ND
011	-		<0.3
012	-		0
013	-		0
014	-		<10
015	-		<10
016	-		<10
017	-		ND
018	-		<10
019	-		<20
020	-		<10
021	+	C_1	0
022	-		<10
023	-		<10
Recovery Rate	1/14		<10

+ Isolated
- Not Isolated

ND Not Detected

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Table 6: Participants' results and intended result for sample 0204

Lab No.	*Salmonella*		Enterobacteriaceae cfu/g
	Isolated	Serogroup	
Intended	**+**	**C_1**	**6.8×10^4**
010	+	C_1	6.9×10^3
011	+	C_1	$>1.1 \times 10^4$
012	+	NR	3.3×10^4
013	+	NR	3.1×10^5
014	+	C_1	4.0×10^4
015	+	NR	8.3×10^4
016	+	NR	8.6×10^4
017	+	NR	6.8×10^4
018	+	NR	5.9×10^4
019	+	C_1	0.9×10^1
020	+	B	2.0×10^3
021	+	C_1	7.4×10^3
022	+	NR	2.8×10^3
023	+	C_1	1.7×10^4
Recovery Rate	14/14		Mean: 6.0×10^4

- Isolated NR Not Reported

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3. DISCUSSION

Salmonella Isolation

All laboratories participating in this ring trail successfully isolated salmonellas from samples 0201 and 0204 which contained approx. 50,000 cfu/g.

Samples 0202 and 0205 were identical and contained *Salmonella* at approx. 5 cfu/g. Only one of the participating laboratories (Lab. No. 019) failed to isolated *Salmonella* from both samples. Lab Nos. 017 and 022 failed to isolate *Salmonella* from sample 0202.

The Quality Control Department isolated salmonellas from all postal return samples and from all 20 low level *Salmonella* samples tested during the distribution period. Future ring trials will demonstrate more clearly whether the laboratories involved (Lab. Nos. 017, 019 and 022) are able to recover low numbers of salmonellas. The Organisers will contact these laboratories and discuss their *Salmonella* isolation procedures.

Cross-Contamination

Only one laboratory (Lab. No. 021) isolated *Salmonella* from the *Salmonella*-negative control sample 0203 and this would seem to be the result of cross-contamination in this laboratory.

Serotyping

Seven laboratories serogrouped their *Salmonella* isolates, but only four of these (Lab. Nos. 011, 014, 019 and 023) reported the intended serotypes. Lab. No. 020 reported group A *Salmonella* and this laboratory should quality control its agglutination sera. Lab. No. 010 mis-identified the *Salmonella* serogroup in sample 0205 and Lab. No. 021 reported the same serogroup (C_1) in all samples, suggesting a cross-contamination or reporting problem.

Enterobacteriaceae Counts

Figure 1 shows that five laboratories (Lab. Nos. 010, 012, 019, 021 and 022) reported outlying Enterobacteriaceae counts for sample 0201.

Figure 2 shows that five laboratories (Lab. Nos. 010, 019, 020, 021 and 022) reported outlying Enterobacteriaceae counts for sample 0204.

Laboratory No. 019 reported extremely low counts in both these samples which may be due to abuse of samples during transit or reflect a problem in methodology.

Sample 0203 contained less than 10 cfu/g Enterobacteriaceae. Results were reported as not detected, 0 or were left blank. The correct reporting would be either MPN counts (eg. <0.3/g) or, if standard plate count methods were used, <10 cfu/g.

4. COMMENTS

This first ring trial organised on behalf of IFOMA, shows that all laboratories were able to isolate Salmonella from samples of fish meal contaminated at the 50,000 cfu/g level.

The failure of laboratories to isolate Salmonella contamination at a lower level may reflect uneven distribution of the organism in the test samples although the method of preparation and results of the Quality Control Department work would suggest that this is unlikely.

There appears to be some major problems with serotyping of Salmonella but this may be due to different international definitions of serotyping procedures.

The range of counts recorded for Enterobacteriaceae is of some concern, especially where data produced by laboratories may be used for interpreting acceptance to export certification agreements.

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In terms of reproducibility, the results found by the Quality Control Department show acceptable scatter, but as expected, when intra-laboratory variations are introduced, the range of counts found increases significantly. Of special interest are the low readings found by laboratories for both samples 0201 and 0204 and again this may be due to difference in methodology. An examination of methods used by participants will be made and a further report presented.

Future trials will examine the potential for intra-laboratory difference when samples with low Enterobacteriaceae counts are used, as these will be at the borderline for international standards.

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