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International Fishmeal & Oil Manufacturers Association

CURRENT SALMONELLA SAMPLING AND ANALYTICAL TECHNIQUES IN MEMBER COUNTRIES

by

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As a result of a questionnaire sent from the Association to all Members and Associate Members asking for copies of current sampling techniques and analytical methods used for determining Salmonella in fish meal, we received responses from twenty organisations representing nine countries and one international organisation. Most sent us both sampling plans and analytical techniques but some only provided one or the other, as shown below.

	Organisation	Source of Information	Sampling Technique	Analysis Technique
Chile	Fundacion Chile	Fundacion Chile	✓	✓
Chile	Corpesca	Corpesca	x	✓
Chile	Sernap	Itata	①	①
Chile	Sernap	Corpesca	x	✓
Chile	Alex Stewart	Alex Stewart	✓	✓
Peru	SGS	SGS	x	✓
Peru	Itintec	SGS	✓	✓
Peru	Inassa	Inassa	✓	✓
Denmark	Aller Mølle	Biotechnological Institute	✓	✓
Germany	Govt Legislation	FMC	✓	✓
Norway	Norwegian Herring Meal Control	SSF	✓	✓
Iceland	Icelandic Fisheries Laboratory	Icelandic Fisheries Laboratory	✓	✓
South Africa	Government	FIRI	x	✓
South Africa	FIRI	FIRI	✓	✓
Switzerland	Govt Legislation	FMC	✓	x
U.K.	BOCM Silcock	Fulmar	x	✓
U.K.	B P Nutrition	B P Nutrition	✓	✓
U.K.	Government	UKAFMM	✓	✓
U.K.	Govt Legislation	IAFMM	✓	✓
International	Codex	FAO	✓	x

① Recommended procedures for sanitary control in plant and during transport.

Sampling

The need to obtain good representative samples is clearly set out in Guidelines for the Control of Salmonella in Fishmeal (IAFMM Processing Bulletin No. 3 July 1990). These guidelines describe sampling plans which are in use in the UK but also refer to other statistical plans. The need for extensive sampling becomes obvious when it is appreciated that Salmonella infection is in small pockets of the meal and thus even if many samples are taken it is possible to miss these pockets. The only way to be absolutely sure that a product is free of Salmonella is to sample and analyse every gram. However that is obviously unrealistic and suitable compromises have to be found.

Sampling plans are identified for nine countries but there is considerable variation as to methods for obtaining representative samples. This present survey shows that the majority of sampling plans are generally sound and refer to the collection of materials from throughout a lot or production batch. Whilst accepting that no sampling plan can be perfect we have no problem with most of the sampling plans each being as good as the other. The Swiss sampling plan requires an unusually low number of incremental samples.

Commonly 7.5%, 10% or square root of bags are selected or the equivalent measure in bulk.

The sampling plans are summarised in Table 1.

Differences are neatly illustrated by comparing plans used in Chile and U.K. In Chile samples are collected for each 50 tons lot taking 100 incremental samples throughout the lot from normal bags. These samples are composited to make one sample for analysis. Thus for 200 tons this would result in 400 incremental samples yielding four composites for individual analysis giving four results.

In U.K. 200 tons would yield 63 incremental samples yielding four composites but each examined in duplicate giving eight results.

Thus in U.K. there would be eight results compared with four in Chile increasing the risk of finding Salmonella in U.K. However in Chile the larger number of incremental samples increases the risk of identifying unevenly distributed pockets of contamination.

If 500 tons of fish meal were regarded as the consignment Chile would have ten results whereas U.K. would still be eight which is the maximum if the consignment is over 40 tons.

The number of question marks (?) on the table represent areas of uncertainty with no clear statements being made in the sampling plans published as government legislation or as industrial guidelines.

TABLE 1
Revised January 1995

SIZE CONSIGNMENT (TONNES)	COUNTRY	N° INCREMENTAL SAMPLES	N° TEST SAMPLE S	TEST SAMPLE SIZE g/sample	TESTING IN DUPLICATE (OR MORE)
50	CHILE	25-100	1	25	NO
	PERU	75-300	1	1,000	NO
	DENMARK	3/24 HOURS	3	?	?
	GERMANY	20	4	100-125	NO
	NORWAY	120	1	1,000	5
	ICELAND	100	1	250	NO
	S. AFRICA	100	1	100	?
	SWITZERLAND	10	?	?	?
	U.K.	32	4	500	YES
200	CHILE	100-400	4	25	NO
	PERU	300-1,200	4	1,000	NO
	DENMARK	N/A	N/A	?	N/A
	GERMANY	80	16	100-125	NO
	NORWAY	480	1	1,000	5 or 8*
	ICELAND	400	2	250	NO
	S. AFRICA	400	?	?	?
	SWITZERLAND	25	?	?	?
	U.K.	40	4	500	YES
500	CHILE	250-1,000	10	25	NO
	PERU	750-3,000	10	1,000	NO
	DENMARK	N/A	N/A	?	N/A
	GERMANY	200	40	100-125	NO
	NORWAY	500	1	1,000	10 or 20*
	ICELAND	1,000	5	250	NO
	S. AFRICA	1,000	?	?	?
	SWITZERLAND	40	?	?	?
	U.K.	40	8	25	YES

*Depending on each factory's Salmonella record

Analytical Methods

Salmonella detection methods were developed based on clinical requirements for patients suffering from food poisonings. In order to confirm the Salmonella infection samples of faeces from the patient were taken which contained large quantities of actively growing Salmonella organisms and many million of other organisms. In order to clearly identify the Salmonella organisms it was necessary to subject the total microbial population to incubation in selected media which were designed to kill off the other organisms and prevent them interfering in the subsequent plating-out and visual identification of Salmonella colonies. Thus the selective media were designed to kill off non-Salmonella bacteria. Because of the nature of these media Salmonella themselves were destroyed to some extent although the presence of such large numbers in the patients' faeces resulted in sufficient surviving to be identified.

When this technique was applied to dried products the Salmonella present in these products was in a dormant state because of the low water activity of the products. If such Salmonella were placed directly into the selective media there was a high probability that the majority, if not all, would be killed by the aggressive nature of this media. Thus the product would be assumed to be Salmonella free. Therefore international bodies introduced a pre-enrichment technique during which the dormant Salmonella could be reawakened and start to multiply. This would supply sufficient numbers with sufficient vigour to withstand the aggressiveness of selective media.

Details of the two internationally recognised methods for Salmonella determination, FDA and ISO, are given in Table 2.

Isolation methods required by the regulatory authorities are shown in Table 3. Some of these are based on the ISO or FDA methods, but often these methods have been modified and this can be quite important. Germany and South Africa have developed their own regulatory methods.

Methods differ in respect of pre-enrichment media, selective media, volumes of media transferred and incubation temperatures. A common element is the volume of 25 g sample examined.

The importance of standard methods cannot be overstated. Many surveys have shown that even minor variations can influence the ability to detect Salmonella. This is particularly true for incubation temperatures of the selective media and in the case of 43°C this has been shown to be too high resulting in failure to recover Salmonella (Rhodes et al 1985; Peterz et al 1989). The temperature of an incubating oven is difficult to control and therefore extreme care should be exercised when using the recommended temperature of 42°C to ensure it is not exceeded.

The International Standard method which recommends Rappaport Vassiliadis (RV) selective medium states that 0.1 ml of pre-enrichment media should be added. The Chilean authorities define 1.0 ml - this is too much. RV is not so aggressive as other selective media for killing other bacteria which interfere with the Salmonella assay. At a ratio of 1 : 10 (rather than the recommended 1 : 100) other bacteria will survive and

TABLE 2

STANDARD METHODS

METHOD	SAMPLE	PRE-ENRICHMENT	TRANSFER VOLUME	SELECTIVE MEDIA/TEMPERATURE	
FDA	25 g	LB 225ml	1.0 ml	TB(10ml)/35°C	SC(10ml)/35°C
ISO 6579	25 g	BPW 225ml	0.1 ml 10.0 ml	RV(10ml)/42°C SC(100ml)/37°C	

LB: Lactose Broth

RV: Rappaport Vassiliadis

TB: Tetrathionate Broth

BPW: Buffered Peptone Water

SC: Selenite Cysteine

confuse the reading of the plated colonies (Quail et al 1986; Fricker et al 1985).

It is now widely accepted that two different selective media should be used for these tests and the FDA and ISO methods incorporate this requirement. Danish, Iceland and the UK methods would not meet this criteria, but the use of a single media is common practice for cost purposes.

The German and South African regulatory methods appear to have significant shortcomings compared with the standard methods.

The use of Malthus technology is now gaining wide acceptance and UK, Denmark and Norway acknowledge this technology. The method also now has AOAC and BSI approval and incorporates the requirement of two different selective media.

Industry Methods

Differences also exist in industry methods (Table 4) and some of the comments already made on the regulatory methods equally apply to the industry methods.

It is interesting that not many observe the recommended government method. In some cases there are only minor variations (SGS[Peru] and UK) but in others significant differences are seen.

In summary, whilst the sampling methods in general use seem acceptable, there is too much variability in isolation methods. Modifications to standard methods will affect results. The FDA, ISO or Malthus methods are recommended but the fully defined standard method should be adhered to and not modified unless clearly documented validation data is available to support any changes. The Nordic method also appears to be a satisfactory method but the absence of two selective media must be noted.

TABLE 3

REGULATORY METHODS

COUNTRY	METHOD	SAMPLE	PRE-ENRICHMENT	TRANSFER VOLUME	SELECTIVE MEDIA/TEMPERATURE	
CHILE	ISO (MOD)*	25 g	BPW 225 ml	1.0 ml	RV(10ml)/42°C	SC(10ml)/37°C
PERU	FDA, (MOD)*	25 g	LB 225 ml	1.0 ml	TB(10ml)/35°C	SC(10ml)/43°C*
DENMARK	NORDIC	25 g	BPW 225 ml	0.1 ml	RV(10ml)/42°C	
GERMANY	OWN	25 g			SC(125ml)/37°C	or TB/37°C
ICELAND	FDA (MOD)*	25 g	LB 225 ml	1.0 ml	SC(10ml)/35°C	*
S. AFRICA	OWN	28-35 g	BPW 252-315 ml+	25 ml	TB(100)/43°C	SC(100ml)/37°C
NORWAY	MALTHUS	25 g	BPW 225 ml	0.1 ml	SC(10ml)/37°C	LD(10ml)/37°C
UK	ISO (MOD)* OR	25 g	BPW 225 ml	0.1 ml	RV(10ml)/42°C	*
	MALTHUS	25 g	BPW 225 ml	0.1 ml	SC(5ml)/37°C	LD(5ml)/37°C

* Modifications to Standard Method

\+ stands 2-6 hours instead of 18 hours

MOD: Modification of Standard Method
LB: Lactose Broth
RV: Rappaport Vassiliadis

BPW: Buffered Peptone Water
SC: Selenite Cysteine
LD: Lysine Decrboxylase

TABLE 4

INDUSTRY METHODS

COUNTRY	LABORATORY	METHOD	SAMPLE	PRE-ENRICHMENT	TRANSFER VOLUME	SELECTIVE MEDIA/TEMPERATURE	
CHILE	1	FDA, (MOD)*	25 g	BPW* 225 ml	1.0 ml	SC/43°C*	
	2	ISO, (MOD)*	25 g	BPW 225 ml	1.0 ml	SC(10ml)/37°C	RV(10ml)/42°C
	3	FDA	25 g	LB 225 ml	1.0 ml	SC(10ml)/35°C	TB(10ml)/35°C
PERU	4	OWN	25 g	TSB 225 ml	1.0 ml	SC(10ml)/37°C	
	5	FDA	25 g	LB 225 ml	1.0 ml	SC(10ml)/35°C	TB(10ml)/35°C
DENMARK	6	NORDIC	25 g	BPW 225 ml	0.1 ml	RV(10ml)/42°C	
S. AFRICA	7	OWN	25 g	BPW 225 ml	10 ml	SC(10ml)/37°C	RV(10ml)/41°C
NORWAY	8	NORDIC	25 g	BPW	0.1 ml	RV(10ml)/42°C	
UK	9	ISO(MOD)*	10 g*	BPW	0.1 ml	RV(10ml)/41°C*	
	10	MALTHUS	25 g	BPW	0.1 ml	SC(5ml)/37°C	LD(5ml)/37°C
	11	MALTHUS	25 g	BPW	0.1 ml	SC(5ml)/37°C	LD(5ml)/37°C

* Modification to the Standard Method

MOD: Modification of Standard Method
LB: Lactose Broth
RV: Rappaport Vassiliadis
TSB: Tryptone Soya Broth

BPW: Buffered Peptone Water
SC: Selenite Cysteine
LD: Lysine Decrboxylase
TB: Tetrathionate Broth