Influence of sample sizes and processing of milk powder for the isolation of Salmonellae

G. Hahn, U. Schleert

Institute for Hygiene, Federal Dairy Research Centre, Kiel

1 Introduction

For the isolation of Salmonellae from milk powder normally 25 g samples should be examined. This results in an enormal need of time and especially of material in routine analysis. Therefore, it is permitted by different standard methods to pool samples. Of course, the pre-enrichment solution (double buffered peptone water-PW) always has to be used in a tenfold amount independent of the size of samples. However, it is not indicated up to how many multiple samples may be examined simultaneously. Therefore, an examination was done to find out the effect of pooling on the isolation rate of Salmonellae.

2 Materials and methods

The basic material for these examinations was a naturally contaminated skim milk powder of one day-lot of 20 tons which was made available to us from a dairy plant. The advantage is in comparison to experimentally contaminated powder that you will have the typical clusters of Salmonellae which makes the isolation and the statistics so difficult. But on the other hand you have realistic conditions as they appear in routine analysis. The material we used were 6 bags (A-F) à 25 kg from the mentioned lot.

For one run 3 kg were taken and divided in different groups of sample sizes a follows: 1 x 750 g, 3 x 250 g, 6 x 125 g and 30 x 25 g each corresponding to a batch of 750 g. Additionally, the original powder was „diluted“ 1:2 and 1:4 with Salmonella-free powder which, however, had a high contamination flora of 3.4×10^3 - 2.3×10^4 cfu/g.

The isolation procedure was a simplified standard method which has proved since years to be as effective:

- Resuscitation of the sample (1:10) in double-buffered peptone water, 8-24 h at 37°C;
- subculturing of 0,1 ml in 10 ml Rappaport-Vassiliadis medium, 24 h at 43°C;
- subculturing by streaking of XLD agar, 18-24 h at 37°C;
- subculturing of subspicious colonies on blood agar, 18-24 h at 37°C;
- confirmation by bacteriological and/or serological (polyvalent sera) and/or biochemical (API 20E) tests

3 Results

By a modified MPN method Salmonellae counts could be detected up to 22,5/500 g.

Table 1 shows the positive results in the original milk powder from the different bags A-F from three independent examinations.

Table 1: Number and percentage of Salmonella positive samples from three independent examinations of original skim milk powder

bag	750 g n = 3	250 g n = 9	125 g n = 18	25 g n = 90
A	3	7	13	12
B	3	7	10	15
C	3	8	9	23
D	3	7	13	26
E	3	6	9	11
F	3	6	4	7
pos./n	18/18	41/54	58/108	94/540
% pos.	100	75.9	53.7	17.4

It is to be seen that the positive scores increase according to the size of sample, i.e. the 750 g samples show the best results. The 17,4 % positives of the 25 g samples, however, do not mean, that the probability to detect Salmonella is only as low. The 30 small samples all are from one batch, which therefore has to be discarded, even if only one of the 30 samples would be positive. On the other hand, however, the results show that it is a professional error to examine only one 25 g sample from a lot as it is, unfortunately, done by some investigators. Normally, nowadays skim milk powder, especially high heat powder, has a very low contamination flora of only some hundreds/g. To see the influence of low Salmonella numbers and a higher contamination flora the original powder was diluted with „dirty“ powder 1:2 and 1:4.

Table 2 shows the results of a 1:2 diluted powder from 3 independent examinations per bag. The principle relation according to the size of samples was comparable to the results of the original powder, but on a lower level. Even two batches (750 g in bag F and 125 g in bag A) showed false negative results in all repetitions.

This tendency continues in the 1:4 diluted powder as it is to be seen from Table 3.

These results show as it was expected at least theoretically that the positive results decrease depending on the decreasing size of the sample and additionally on the dilution. A statistic evaluation cannot be done due to the few numbers of data.

An evaluation can be done, however, by the following aspects:

- All bags contain Salmonellae;
- the total of 3 kg as a basic material for one examination splitted in the mentioned sample sizes may to be seen as one independent „lot“ as it could be in practice;
- for every of this lots 750 g have to be investigated by one of the mentioned sample size models;
- the lot has to be refused if only one sample within the lot is Salmonella positive;
- in these investigations therefore 18 lots were available
  (3 independent examinations of 6 bags).

Table 2: Number and percentage of Salmonella positive samples from three independent examinations of 1:2 diluted skim milk powder

bag	750 g n = 1	250 g n = 3	125 g n = 6	25 g n = 30
A I	0	2	0	2
A II	1	1	0	2
A III	0	0	0	1
B I	1	0	1	4
B II	1	1	1	1
B III	1	1	3	1
C I	1	2	1	3
C II	1	2	0	1
CIII	1	1	2	1
D I	1	3	1	2
D II	1	2	3	2
D III	1	1	0	3
E I	1	0	0	3
E II	1	3	0	0
E III	0	2	2	3
F I	0	1	3	1
F II	0	1	0	2
F III	0	1	1	0
pos./n	12/18	25/54	18/108	32/540
% pos.	66.7	44.4	16.7	5.9

Table 3: Percentage of Salmonella positive samples depending on sample size and dilution

milk powder	750 g	250 g	125 g	25 g
original	100 %	75.9 %	53.7 %	17.4 %
1:2	66.7 %	44.4 %	16.7 %	5.9 %
1:4	44.4 %	25.9 %	16.7 %	4.4 %

Under this aspect the Salmonella positive lots depending on the dilution and sample size are summed up in Table 4.

Table 4: Percentage of Salmonella positive „lots“ depending on sample size and dilution

milk powder	750 g	250 g	125 g	25 g
original	100 %	100 %	100 %	100 %
1:2	66.7 %	83.3 %	55.6 %	88.9 %
1:4	44.4 %	55.6 %	72.2 %	72.2 %

On this basis, consequently, all lots of the original powder had to be assessed as Salmonella positive, independent of the sample size, but on condition that the total of 750 g is examined by one of the splitting models. The second best score of 88.9% was with the 25g samples.

For the transfer of these results to routine analysis the following recommendations could be made:

For the assessment of one lot of milk of high quality concerning the reliability and the enormous saving of material and processing time it may be recommended to examine a single batch of 750 g. In this case it is necessary to use about 10 l flasks for the 7,5 l volume of pre-enrichment and shake them several times during incubation.

If you have milk powder of less quality the examination of 30 x 25 g per lot seems to give better results regarding the total lot.

Accompanying to this trials another question was dealt with: according to some standard methods IDF, ISO, LMBG § 35 (1, 5, 6) for some substrates it is allowed to use distilled water or a salt solution for pre-enrichment instead of peptone water. The background of this is that the substrate milk powder itself contains enough of amino acids, oligo peptides etc.

The results of a comparison is shown in Table 5:

Table 5: Comparison of different pre-enrichment (resuscitation) solutions for the isolation of Salmonellae (positive samples)

solution	milk powder	
	original	1:2
	n = 30	n = 30
distilled water	14	2
salt solution	13	0
peptone water	13	3

Since years we, therefore, use a double buffered salt solution corresponding to the double buffered peptone water but without the peptone itself.

4 Discussion

The main question of this investigation was to see the relation of the multiple number of pooled 25 g milk powder samples and the efficacy of positive Salmonella isolations. The aim could be saving enormously material and time for the examination, when e.g. the FOSTER plan (3) has to be carried out. Simular investigations were done by others (2,7,8).

Our results show that an optimal recommendation is depending on the quality of milk powder. For a high heat powder which usually contains a very low contamination flora of only some hundreds/g it is obvious to use the biggest batch of 750 g in 7.5 l pre-enrichment solution in a 10 l vessel. For worse powder with a high contamination flora and less Salmonellae the examination of 30 x 25 g per lot seems to show better results. But these kinds of powders are very rare in food hygiene. The reason we used the 750 g batch as the biggest amount may be explained by the FOSTER plan. There you need for the highest quality level 60x25 g per lot, which means an amount of 1.5 kg. In many dairy

plants, nowadays, autosamplers are used according to Habraken et al. (1986), which deliver aliquots of about 2.5 g during the drying process for test samples. By this a much higher chance to detect Salmonellae is given. Therefore statistically the 750 g batch is sufficient.

The additional examination to save a lot of material and money is the use of a salt solution or distilled water for resuscitation of milk powder. Both modifications are real improvements to simplify the isolation procedure of Salmonellae detection.

5 Summary

The aim of these examinations was to see the effect concerning the detection of Salmonellae by different pooling of milk powder. In three independent trials for each examination 3 kg of naturally contaminated skim milk powder were taken and divided into batches of 1x750 g, 3x250 g, 6x125 g and 30x25 g. The original powder additionally was "diluted" 1:2 and 1:4 using heavily contaminated but Salmonella-free powder containing a contamination flora from $3.4x10^3$ - $2.3x10^4$ cfu/g. The isolation procedure was done by a modification of the usual international standards. In the original powder from all 750 g samples (n = 18) (6 bags and three independent investigations) Salmonellae could be isolated (100%). From the same batch from 540 (6x90) 25 g samples only 17.4% were positive. In the 1:2 powder the relation of results were comparable but on a lower level. This continued with the 1:4 dilution. However, it is to emphasize that not only e.g. 17.4% of Salmonellae were detected. In principle, according to the FOSTER plan 30x25 g has to be examined for one lot. If only one of these is positive the total lot has to be refused. Therefore the results have to be interpreted regarding not the single sample than the total lot. Additionally, the effect of replacing peptone water for resuscitation in milk powder by salt solution or distilled water was investigated. The results were comparable which enables to save a lot of expenses. A recommendation for a more material and time saving method is made.

References

1. Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG: Nachweis von Salmonellen (L02.00-8/L00.00-20).
2. Becker, H.: Analyse von Sammelproben und Enterobacteriaceae als Index-Mikroorganismen – Ein Beitrag zur Rationalisierung der Untersuchung von Trockenmilchprodukten auf Salmonellen, Vet. Med. Dissertation, München 1981.
3. Foster, E.M.: The Control of Salmonellae in Processed Foods: A Classification System and Sampling Plan, Journal of the AOAC, 54 No. 2, 259-266 (1971).
4. Habraken, C.J.M., D.A.A. Mossel and S. van den Reek: Management of Salmonella risks in the production of powdered milk products, Neth. Milk Dairy J. 40, 99-116 (1986).
5. International IDF-Standard 93 A:1985 – Milk and Milk Products – Detection of Salmonella.
6. International Standards: Milk and Milk Products-Detection of Salmonella, Ref. No. ISO 6785-1985 (E).
7. Price, W.R., Olsen, R.A. and Hunter, J.E.: Salmonella Testing of Pooled Pre-Enrichment Broth Cultures for Screening Multiple Food Samples, Appl. Microbiol., 23, 4 679-682 (1972).
8. Silliker, J.H. and D.A. Gabis: ICMSF methods studies. I. Comparison of analytical schemes for detection of Salmonella in dried foods. Can. J. Microbiol. 19: 475-479 (1973).