THE CONTROL OF *LISTERIA* CONTAMINATION IN THE FOOD INDUSTRY *

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SUMMARY

The control of Listeria contamination has many points in common with the control of any microbial contamination except that it is probably much more tenacious than the majority of microorganisms.

Dividing a factory into zones of different hygienic quality, efficient separation of these zones and elimination of vectors are the measures to be applied along with efficient cleaning and disinfection and elimination of unecessary wet areas and points of residue build-up.

In cases where a problem has not been foreseen, sampling of the factory should be carried out and the points of contamination identified. The critical points are those that have direct contact with the product. Successful control is characterised by reduction in contamination which follows an exponential curve. These concepts of control are illustrated using the example of Listeria in frankfurter production.

RESUME

La maîtrise de la contamination par Listeria a de nombreux points en commun avec celle de toute contamination microbienne, mais cette contamination est probablement plus tenace que celle de la majorité des micro-organismes.

La division d'une usine en zones de qualité hygiénique différente, la séparation efficace de ces zones et l'élimination des vecteurs sont les mesures à appliquer en même temps qu'un nettoyage et une désinfection efficaces ainsi qu'une élimination des zones humides non nécessaires et des points d'accumulation de résidus.

Au cas où un problème n'aurait pas été prévu, des prélèvements devraient être faits dans l'usine et les points de contamination identifiés. Les points critiques sont ceux qui ont un contact direct avec le produit. Une maîtrise efficace est caractérisée par une réduction de la contamination qui suit une courbe exponentielle.

Ces concepts de maîtrise sont illustrés en prenant l'exemple de Listeria dans la production de saucisses de Francfort.

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I - INTRODUCTION

Listeria monocytogenes has been recognised as a problem since outbreaks in the early 1980's conclusively proved Listeriosis to be a food-borne disease. Although the disease is relatively uncommon it is nevertheless extremely serious and for food manufacturers it is a disaster for one of their products to be implicated in an epidemic.

In the early 80's the industry was caught unawares and at that time we had to work very quickly and very hard to find out exactly with which processes and products this organism (and its non-pathogenic relatives) were associated.

In our company this resulted in a publication [Cox *et al.*, 1989] which showed that this organism was quite commonly associated with the environments of most food factories (and even some non-food environments) except those producing dried foods from dry raw materials. These results are summarised in table 1.

The Food Industry has now much experience in the control of *Listeria* contamination in many different food processes. However, the experience gained has not often been passed on through the scientific literature. In this article we shall illustrate some of the principles involved in control using the example of a four year study we have made of frankfurter processing.

II - FOUR YEAR FOLLOW-UP OF FRANKFURTER PRODUCTION

A - GUIDE TO ENVIRONMENTAL CONTROL

Zaika *et al.* [1990] has shown that the frankfurter process is a safe process with regard to the destruction of *L. monocytogenes*. For this reason we shall not go into the process itself. Manufacturer should be able to set experimentally the criteria for heat-processing of a product so that the most resistant pathogens expected to be present are killed.

For a manufacturer realising he has a microbiological problem about which he knows little there is a set of clear steps to take in order to assess the magnitude of the problem and where to attack it to best effect. Since this process is retrospective (i.e. the problem already exists) we cannot strictly speaking call it HACCP. However, the steps are essentially the same.

These are shown in table 2 and they are carried out with the aid of the flow diagram of the process in the production area itself.

Table 1 : Occurrence of *Listeria spp.* in Food Processing Environments

SAMPLING POINT	TYPE OF FACTORY*									
	1	2	3	4	5	6	7	8	9	10
DRAINS / EFFLUENTS	22#	71	80	66	100	37	0	-	50	53
FLOORS	20	5	83	-	63	29	0	-	0	75
CONDENSATION	5	-	-	-	-	7	0	-	(	-
STAGNANT WATER	14	-	-	-	66	-	0	-	(40	83
RESIDUES (WET)	-	23	46	-	50	-	0	-	-	50
EQUIPMENT (FOOD CONTACT)	0	0	-	15	75	20	0	-	0	0
RESIDUES (DRY)	-	-	-	-	-	-	0	-	-	50
WALLS	33	-	-	-	-	(	0	-	-	-
CEILINGS	0	-	-	-	-	(5	0	-	-	-
DAMP/WET AREAS	-	-	-	-	-	-	0	25	-	-
CLEANING AIDS	-	-	-	-	-	24	0	-	-	-
WASH AREAS	-	-	-	-	-	24	0	-	-	-
SAUSAGE PEELERS	-	-	-	-	-	22	-	-	-	-
AIR COOLERS	22	0	-	-	-	-	-	-	-	-
DRY AREAS	-	-	-	-	-	-	0	-	-	-
COMPRESSED AIR	-	-	-	-	-	4	-	-	-	-
BRINES	-	0	-	-	-	-	-	-	-	-
MISCELLANEOUS	19	-	-	20	-	-	-	-	8	-

**1, Soft cheese ; 2, Blue cheese (production) ; 3, Blue cheese (ripening) ; 4, Frozen foods ; 5, Ice cream ; 6, Meat (data of American Meat Institute) ; 7, Dry culinary products; 8, Cocoa ; 9, Dairies ; 10, Potato processing.*

 # = % positive samples.

Table 2 : Steps to follow for assessment of the hygienic status of a factory.

1. Zone factory	• Environment remote from product (non-contact, non-critical areas) • Environment immediately around production line (non-contact critical areas) • Product-contact surfaces (critical) • Product (critical)
2. Identify interfaces between zones	• Ascertain degree of separation • Examine personnel and other traffic between zones • Organise separation of zones and elimination of vectors of contamination. Rationalize personnel traffic.
3. Identify wet areas and points of build-up of residues	• Eliminate/minimise (If methods of examination for organism in question are not rapid this should be done immediately)
4. Identify killing step	• Check that parameters are sufficient
5. Identify point at which surface area/volume of product increases	• Can be major point of contamination or recontamination if down stream from 4.
6. Classify the product according to WHO classification	

The WHO classification mentioned in point 6 above was originally devised in the informal working group on Listeriosis that met in 1988 [WHO (1988)]. However it can be extended in the author's opinion to almost any organism. This classification is seen in table 3.

A full assessment of the risks should include also the type of consumer for which a product is destined (Hospital patients, children, old people, etc.) [ICMSF 1986]. In general, foods in group 3.b.ii. pose a risk when the organism concerned is able either to grow in the gut or other organ and (or?) overcome the natural defences of the human body. Foods of group 3.a.ii generally pose a potential risk for all consumers. Indeed, if we look at most outbreaks of food-borne disease associated with processed foods, most of the foods involved belong to group 3. The author would classify Listeriosis as being mainly related to the consumption of foods in the group 3.a.ii. Frankfurters, soft cheeses, pâtés, products in aspic and other products of cheese makers and charcutiers/traiteurs fall into this group. Salmonellosis may be transmitted by foods in groups 3.a.ii and 3.b.ii. as well as those in groups 1 (raw meats) and 2 (e.g. salami).

However, whether such foods pose a risk or not depends almost entirely on whether the organism in question is present in the food (groups 1-3 in table 3), and this in turn depends on whether it is present in the factory/production area and can gain access to the food (groups 2-4 table 3). Furthermore it requires suitable conditions for growth to occur in the product groups 2-3 (table 3).

Here is where the work starts for the microbiologist.

Table 3 : Classification of foods according to the World Health Organisation (1988)

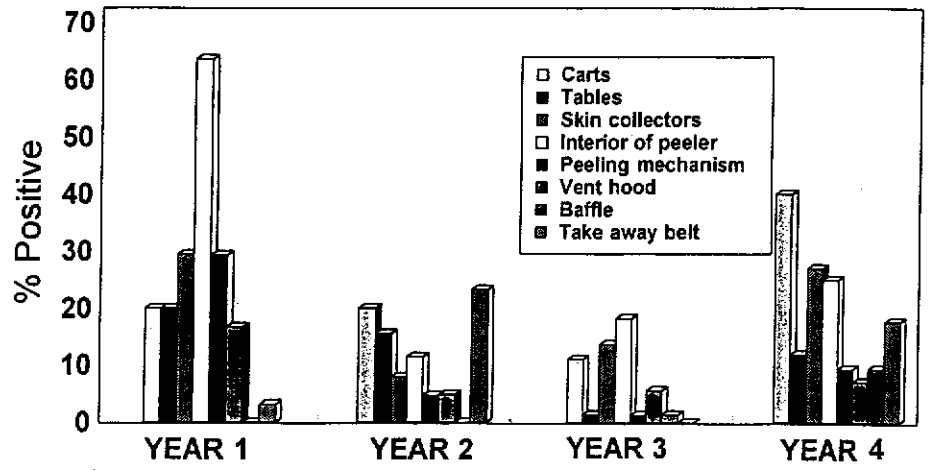
PRODUCT TYPE	INTRINSIC CHARACTER	TREATMENT
1. Raw foods	Always a risk of pathogen presence	
2. Transformed raw foods	a. Products that permit growth of organism	i. Cooked before eating ii. Ready-to-eat
	b. Products that do not permit growth (but permit survival)	i. Cooked before eating ii. Ready-to-eat
3. Processed foods 'cidal process + further handling/ operations after 'cidal process	a. Products that permit growth of organism	i. Cooked before eating ii. Ready-to-eat
	b. Products that do not permit growth (but permit survival)	i. Cooked before eating ii. Ready-to-eat
4. Processed foods 'cidal process in-pack or before aseptic filling	Should not contain any pathogenic organism	

B - INVESTIGATIVE SAMPLING OF THE FRANKFURTER FACTORY ENVIRONMENT

Investigative sampling in the first year of our study revealed certain points that were frequently contaminated with *Listeria*. We were able to profit from our previous experience and only pay attention to wet areas and product contact surfaces that might have some significance due to either insufficient separation from areas that should be dry or to their proximity to the exposed products. In this way we were able to identify the peeling rooms and packing rooms of this particular factory as the areas for greatest attention. This previous experience was very useful at the time, since the *Lustre* methods we had were slow and laborious. We were thus able to direct our resources as efficiently as we could at the time.

Our investigations carried out in the first six months of year revealed the peeling rooms to be a major potential source of contamination. One of the two rooms was particularly important since the sausages arrived into this room directly from the shower of cooling brine. This resulted in the room being wet and thus conducive to microbial growth. The floors we assumed to be contaminated due to our previous experience in similar studies (see table 1). The second room, although not as humid as in the first was open to contamination via the carts used to transport the sausages from the other room. Both rooms were the site of a lot of human activity and thus contamination could be spread from the floors to the equipment and then to the product. In figure 1 are shown the percentages of positive isolates obtained from points in the peeling rooms. Not all the points in figure 1 have direct contact with the peeled sausage.

Figure 1 : *Listeria spp. isolations in Frankfurter peeling process*



The peeling process involves the following operations :

1. Removal of sausages on racks from brine shower.
2. Pose racks on cart to await peeling in room 1 or transfer to room 2.
3. Remove sausages manually from racks.
4. Pose sausage string on table.
5. Introduce string into peeling machine.

Up to this point the sausages are still protected by their skins.

The peeling machine then impels the sausage over a knife that slits the skin. The skin is loosened by steam and then taken away under vacuum to a collection chamber beneath the peeler ; this process is extremely rapid.

THE PEELER MECHANISM IS A CRITICAL POINT

The collection chamber and the support structures are problem points. Although they have no direct contact with the product. They do represent points of undesirable contamination build-up and the workers have contact with them during emptying of the chamber and may serve to spread contamination to the tables thus setting up a cycle of recontamination of the equipment.

The sausages are "shot" from the peeler and hit a baffle that directs them on to the take-away conveyor.

THE TAKE AWAY BELTS ARE ANOTHER CRITICAL POINT HAVING DIRECT CONTACT WITH THE PEELED EXPOSED PRODUCT

Even so, in the first year of the study they were contaminated at a low frequency (3.1%). However, the study revealed that they could be prone to contamination according to what was happening in the peeling rooms.

At that time the peeled sausages were conducted by a take-away conveyor belt to a series of belts to distribute them to the packing machines. These belts are high off the floor, cleaned only with difficulty, and could transport contamination from the peeling rooms to the packing area.

THE ASCENDING BELTS WERE FOUND TO BE A PARTICULAR CRITICAL POINT DURING THE FIRST SIX MONTHS OF THE STUDY.

In view of the time between starting the analysis and the result (at the time several tests were required just to confirm *Listeria spp.* and samples had to be sent to a laboratory 300 km from the factory) no important changes in cleaning and sanitation measures were made during the first six months of the study although points of residue build up (particularly on conveyors) were noted for reference and ways of eliminating them were sought. We needed information concerning the frequency of contamination of the different points because enumeration was not possible at that time (and still is problematic).

From the second six months of the first year hygiene measures were improved. In figures 1, 2 and 3 we can see the effect of these over the four year period. The trend for the two peeling rooms is shown in figure 2. After starting more intensive cleaning and sanitation at the critical points we can see that the trend to the end of the third year was downward. We can see too that if things worsen as they did in the second half of year 2 some time is required before the situation can be returned to normal. It is worth noting however that the increases that are seen in a bi-annual summary tended to be due to periodic problems rather than a continual year-long problem. In figure 1 we can see the progress of the different sampling points in the peeling rooms. We have not separated data for the two peeling rooms. The figure illustrates the difficulty of eliminating contamination completely.

Some points in the process responded immediately to the measures applied. These correspond to points or equipment that are accessible to cleaning and/or not exposed to recontamination on a frequent basis. Notable examples were the distribution belts and the "Hooper" hoppers. Even so, these points could, as seen in figure 3, become recontaminated. Other points showed a slower response. Among these were the ascending belts that were quite complicated and difficult to clean, although in the second year these remained free from contamination.

Figure 2 : *Listeria spp.* isolations in Frankfurter peeling rooms - Four year summary

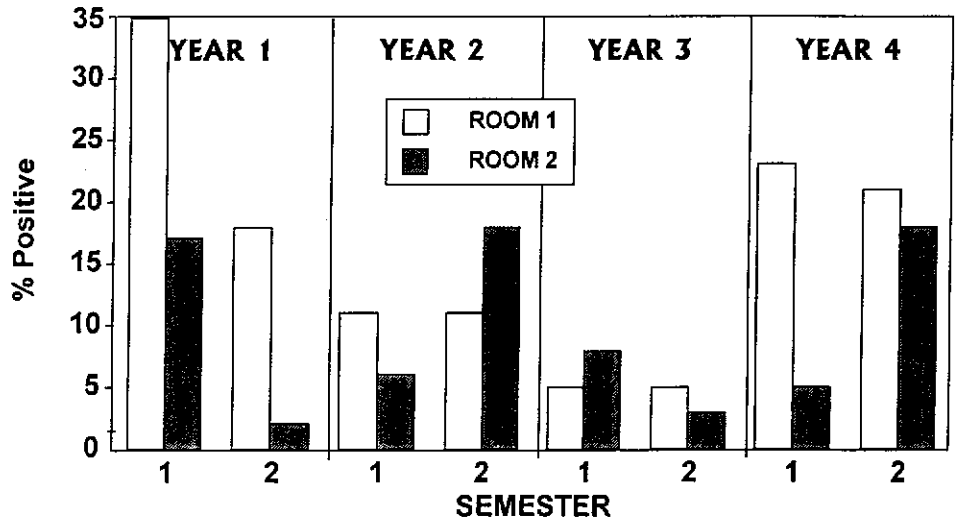
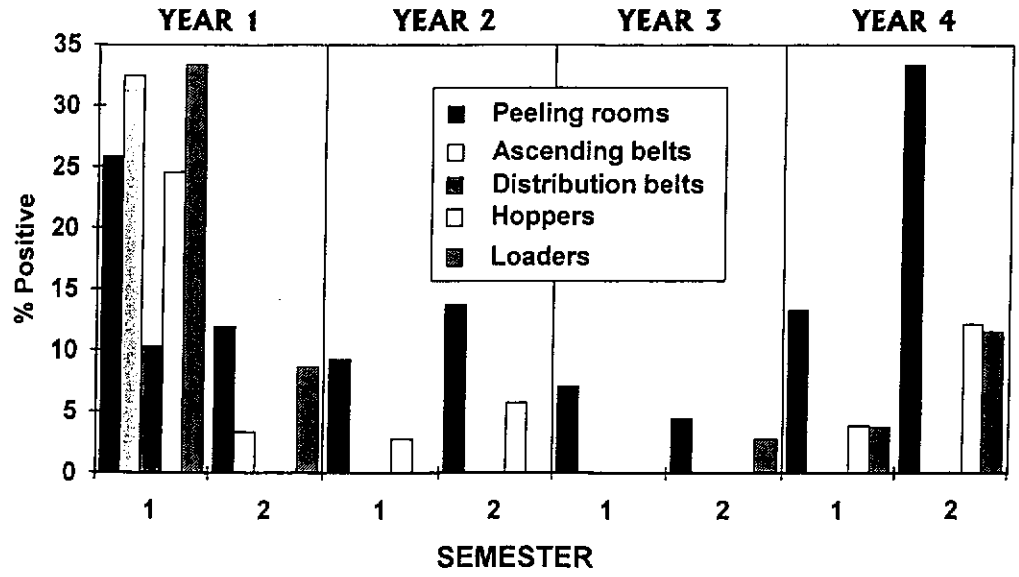


Figure 3 : *Listeria spp.* in Frankfurter production - Four year's monitoring



In the middle of the third year the distribution system in the packing room was phased out and replaced by a mobile "Hooper" hopper filled directly from the take-away belt through the opening in the wall between the peeling room and the packing room. This was because in principle the conveyors still had the potential to be a source of contamination in the packing room and could potentially contaminate large amounts of product. The Hooper could be cleaned in depth daily with less time taken than for cleaning of the belts. With this system too there was the advantage that the majority of the product only touched other product and only a small surface of the product was in direct contact with the hopper.

A full appreciation of the effects of contamination, hygienic measures and the current situation in the peeling rooms can only be gained by analysing the weekly sampling plan over the four years. There is unfortunately not enough space to put this there. However the conclusions we have drawn are as follows.

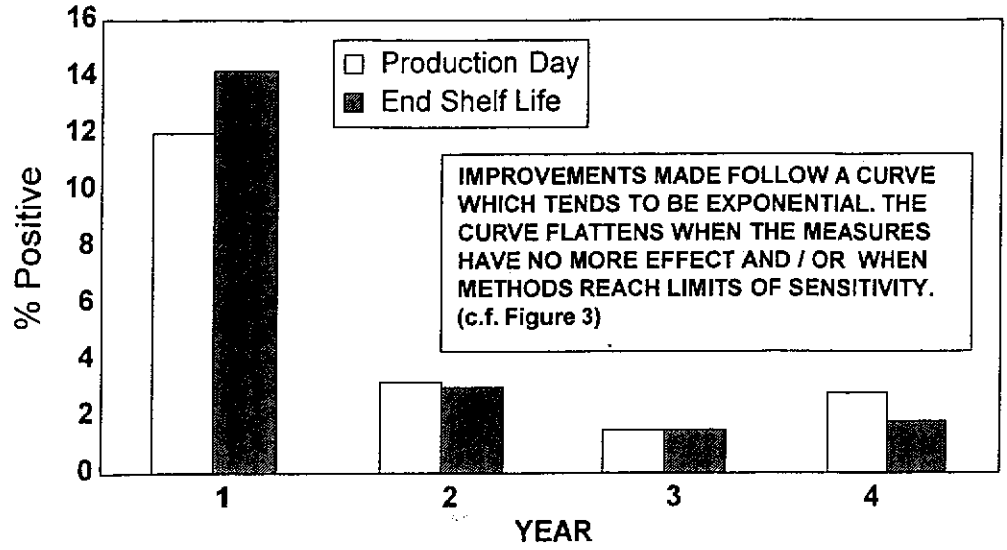
1. There is still the possibility that increased contamination in the peeling rooms could find its way to the packing room. At the end of the fourth year, there was dismantling work going on in the peeling rooms and cleaning could not be carried out effectively. A corresponding increase in isolates was seen in the hoopers and warricks. This can be seen in figure 3.

2. Under such conditions we tend to see an increase in the number of *L. innocua* isolated compared to *L. monocytogenes*. We would tentatively suggest that the former organism might indicate a general deterioration in the hygienic quality of the environment. However, until we study this further we cannot rule out that there might not have been some other factor allowing them to establish on the equipment. In the last year of the study 78% of all isolates were *L. innocua*. However, the overall increase in isolates noted in the last year was not entirely due to the work going on in the peeling rooms. Considering all activities that went on we came to the conclusion that the effectiveness of the disinfectant used may have decreased due to the build up of resistant strains. For this reason we now rotate sanitation agents on a weekly basis. Results in the first months of this year indicate that this conclusion could have some foundation.

C - EFFECT ON PRODUCT CONTAMINATION LEVELS

The overall effect on product can be seen in figure 4. In the first year 12-14% of product contained *Listeria spp* (< 10/g) on the day of production and after the end of shelf life. The measures introduced in the factory had an immediate effect in the second year. In the third year frequencies were further reduced. In the fourth year, despite the problems mentioned in the peeling rooms, the increase in contamination of the product was relatively small. It seems that the peeling rooms, despite their potential problems contributed relatively little to the contamination of the product during this year. Remaining contamination needs therefore to be investigated further to see if it can be reduced. However, the effect one could expect might not be great since the reduction seems to proceed according to a law of diminishing returns. Figure 4 shows an average which reflects sporadic episodes of contamination during each year rather than regular contamination. Contaminated lots were pasteurised in-pack.

Figure 4: *Listeria spp.* in Frankfurters - Four years monitoring



D - REMAINING QUESTIONS CONCERNING HYGIENE MEASURES AND CONTAMINATION

When tracing contamination by organisms such as *Salmonella spp.*, we have the advantage of a very fine discriminatory typing system based on over two thousand different serological patterns. Having such systems is essential to discern the exact sources and routes of contamination in a factory and the degree of success of hygienic measures. Until very recently no system of sub typing other than lysotyping and serotyping has been available and these latter are not very discriminatory. However, new sub typing systems are now becoming available. Among these are RAPD (random amplification of polymorphic DNA) and restriction enzyme analysis (REE) and others that promise alone or in combination to be of great use. Such sub typing is essential if we are to be able some questions that remain. Some of these are given below.

- When cleaning and disinfection is optimal, is the sporadic contamination that remains due to colonisation of inaccessible areas of the equipment (important to answer for the packing room) ? If so how can we eliminate these points?

- How important is the contamination in the peeling room in its relation to that in the packing room ? Here the information will allow us to decide whether we have to make radical changes in the peeling rooms or whether simple measures such as blocking a door or installing a shoe-change area would be sufficient to minimise transfer from the peeling room or communal areas. Do other routes exist ?

- How is the contamination in frankfurter production related to that occurring in other areas of the factory (e.g. raw products such as bacon and salami) ? Are we dealing with strains restricted to this area only. Do strains from the other areas again access, if so how ?

- How are the strains isolated today compared to those isolated at the beginning of the study, the same or different ?

  We are fortunate enough to have kept the majority of isolates made during this period from product and environment and can now subject them to these new sub typing methods. We hope the knowledge this gives us will lead to some innovative cost effective changes in production.

E - CONCLUSIONS OF ENVIRONMENTAL STUDY

Taking our experience from this study along with that from other areas of food production we conclude that several very basic principles can be demonstrated that show the characteristics of contamination problems and thus how to solve them.

- **In the food chain from raw material production to finished product there are points of contamination build-up due to microbial growth. These are primary contamination sources for the food and/or processing equipment downstream. These area can be identified by looking for water and residues and hygienic measures started even before looking for the microorganims concerned.**

  - They are characterised by frequent isolation or isolation in high numbers of the organism in question.

  - In Frankfurter processing in the factory studied the primary sources of contamination were the peeling rooms.

  - **Contamination is spread by vectors from the primary points.**

    . In the case studied the vectors were mainly conveyors belts and to a certain extent personnel.

    . Spread may be limited by separation of the primary contamination sources from areas dealing with the exposed product.

  - **Surfaces in contact with the product are the major source of contamination.**

    . The surfaces in this study were the conveyor belts and loading machines.

  - **Product is most exposed to contamination after the point where the surface area to volume ratio increases (e.g. after slicing or moulding cheeses).**

    . Frankfurters become most exposed after peeling.

  - **Sanitation and cleaning eliminate contamination.**

    . Resistance to sanitation and cleaning generally indicates points that have accessibility problems, but it can indicate inappropriate disinfectants.

  - **We can live with microorganisms in the food processing environment.**

F - CONCERNING MICROBIOLOGICAL METHODS

The methods used for detection of *Listeria spp.* have changed over the time of this study. At the beginning we used an unbuffered enrichment medium and McBride agar. In year 3 we changed to a buffered enrichment medium and PALCAM agar. We also started carrying out analyses at the factory itself. Although we changed the method to a quicker and more sensitive one we did not increase our isolations significantly. However, we were continually improving our hygienic measures so any increase might have been balanced by the overall reduction in contamination. Analysing samples at the factory allowed us to implement a control plan we had devised in year 2 but had not been able to carry out because of the time taken for the method and the fact that the analyses were carried out by another laboratory distant from the factory. Previously it was difficult to monitor critical points more intensively after contamination had been detected (in order to monitor the hygiene measures applied as a result) since the results came one month after the sample was taken.

There are now more rapid gene probe methods available that considerably reduce analysis time (by 3 days). These do however cost from 3 to 5 times more per test (depending on whether genus or species-specific probes are used) than the method we have been using. Their application, however, could be of great benefit when contamination is detected at critical points using the conventional method. Hygiene measures may be stepped up and the points monitored more frequently using the probe method which would allow us to determine quickly when the points are again under control and to the slower cheaper method for baseline monitoring. This is the case as we see it for production of frankfurters. For other more critical products which support rapid microbial growth (e.g. products in aspic) a control program based solely on the rapid method might be the wisest solution.

III - ACKNOWLEDGEMENTS

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IV - REFERENCES

1. COX L.J. et al. (1989).- *Listeria spp.* In Food Processing, Non-Food and Domestic environments. Food Microbiology, **6**, 49-61.

2. ICMSF (1986).- Microorganism in Foods 2. Sampling for microbiological analysis : Principles and specific applications. 2nd Edition (Chapter 5). Choice of Sampling Plan according to purpose. Blackwell Scientific Publications.

3. WHO (1985).- Foodborne Listeriosis. Report of a WHO Informal Working Group. Geneva, February 15-19, 1988.

4. ZAIKA L.L. et al. (1990).- Destruction of *Listeria monocytogenes* during Frankfurter Processing. Journal of Food Protection, **53** 1821.

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