DANISH TECHNOLOGICAL INSTITUTE

Final Report

Bacterial transfer between products and surfaces

Listeria transfer in slicer and on conveyor belt

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Summary

Purpose

If *Listeria monocytogenes* is detected in a sliced product, it is required to recall all products manufactured on that production day (between two deep-cleaning cycles), unless the products are stabilized for growth of *L. monocytogenes* in the entire shelf life. The purpose of this project is to generate input for a risk assessment of how much product should be recalled if *L. monocytogenes* is detected on product or equipment. The goal is to examine and describe how bacteria travel between product and equipment and, if possible, to make a calculation tool that can determine how much product to discard following a contamination incident.

Conclusion

When the method of direct imprint was used, the transfer rate of *Listeria monocytogenes* (*Lm*) between ham and conveyor belt (product-to-belt-to-product) was approx. 2%. However, on a pre-contaminated conveyer belt, *Lm* could still be detected on the belt after 100 imprints with “clean ham”.

Interval cleaning has a significant effect on the levels of *Lm* on both product and conveyor belt and may be considered a good tool to knock down accidental contaminations during shifts.

Slicing on two different slicers gave varying results. This may be partly due to the slight difference in initial contamination level and partly due to small differences in the equipment design.

A one-time contamination incident led to an immediate accumulation in the equipment, which slowly tapered off but never totally disappeared. Even after slicing more than 10,000 slices of ‘clean’ bulk product, *Lm* could still be found in several places (niches) on the equipment. Niches harbouring *Lm* are problematic, as they may be a continuous (although sporadic) source of contamination for a subsequent clean product.

After a low or ultra-low contamination incident, most of the contamination was transferred from the equipment to product within 2,000 slices, to reach a relative equilibrium after that. For the ultra-low contamination incident, which best mimics a ‘real life’ situation, it is noteworthy that after approximately 1,300 slices, the contamination level dropped below the rejection limit of 1 CFU in 25 g. But due to sporadic release from niches, it cannot be ruled out that occasional low-level counts of *Lm* can occur on later slices, unless these niches are cleaned.

Tables were generated, listing the probability of finding more than one *Lm* in 1 gram (or 25 g) in a slice of 'clean' product after a contamination incident. The tables could aid in a dynamic risk assessment and ultimately minimize the waste of product after a confirmed contamination incident. Furthermore, they may provide valuable input to the dialogue with food inspection authorities following an incident.

Introduction

Purpose

The purpose is to examine how long a slicer and a conveyor belt – which is contaminated with *Listeria monocytogenes* (*Lm*) via a product – can transfer Listeria to subsequent non-contaminated products being sliced. It is examined how much product that needs to be processed through the slicer and on the conveyor belt before it is no longer possible to detect Listeria in product and environmental samples (swabbing).

Background

In the meat processing industry, there is a risk of cross-contaminating equipment with pathogens from products and vice versa from equipment to products. There is a special focus on slicing processes and on the risk of cross-contamination of ready-to-eat products with *L. monocytogenes*.

If *L. monocytogenes* is detected in a sliced product, it is required to recall all products manufactured on that production day unless the products are stabilized for growth of *L. monocytogenes* in the entire shelf life (Appendix 0). The aim of this project is to develop a calculation tool that can help the quality managers in the meat industry to decide on how large sections of a day's production that needs to be discarded in case pathogens are detected.

The results of the project are expected to be of help to particularly processors with long production hours and little or no separation into smaller batches. By calculating how much product will be contaminated following a confirmed contamination, the volume of product that must be discarded may be minimized.

Summary of experiments

Summary of experiments

During the project (2019-2020), various experiments were conducted to investigate how bacteria transfer between equipment and product in the slicing process. The experiments have included:

- Imprint experiments
  Determining the rate of bacterial transfer between product and conveyor belt through direct contact, i.e.
  - Contaminating the conveyor belt by placing a spiked product on it, to determine how many cells are transferred from a contaminated product to a clean conveyor belt.
  - Successively pressing multiple pieces of non-contaminated product onto the contaminated conveyor belt to determine the belt-to-product transfer rate.

- Slicing experiments
  Determining the rate of bacterial transfer between sliced product and an industrial slicer through direct contact, i.e.
  - Contaminating the slicer by slicing a spiked bulk product with a clean slicer
  - Subsequently slicing clean bulk product with the contaminated slicer

  In the slicing experiments, the effect of various factors was investigated:
  - Interval cleaning
    - No interval cleaning
    - Interval cleaning (using alcohol wipes)
  - Level of contamination
    - Medium – 5-6 log CFU/g
    - Low – 3-4 log CFU/g
    - Ultra-low – 0.1-1 log CFU/g
  - Type of product
    - Ham
    - Baloney
  - Slicer model
    - Dixie-Union Verpackungen GmbH (1974)
    - Dixie-Union Verpackungen GmbH (1976)

Abbreviations and definitions

The following abbreviations and definitions are used throughout the report:

Abbreviation	Definition
Lm	*Listeria monocytogenes*
CFU	Colony Forming Units
IC	Interval cleaning
NIC	No interval cleaning
M	Medium spiking level (5-6 log CFU/g)
L	Low spiking level (3-4 log CFU/g)
UL	Ultra-low spiking level (0.1-1 log CFU/g)
(1)	Experiment no. 1
(2)	Experiment no. 2
MRD	Maximum Recovery Diluent
APC	Total Aerobic Plate Count
PCA	Plate Count Agar
Lm quant	*Listeria monocytogenes* by quantitative method
Lm qual	*Listeria monocytogenes* by qualitative method (presence/absence)
Lm semi-quant	*Listeria monocytogenes* by semi-quantitative method
n.d.	Not detected
N/A	Not available/applicable

Strains for spiking

For all experiments, a cocktail mixed of equal volumes of five different strains of *L. monocytogenes* was used:
Lm 3012 Px (Serotype unknown, blade slicing of cold cuts, 1997)
Lm 4106 Px (Serotype 1/2a, SVS (SLCC 2371/ATCC 19111) => FE Lm16)
Lm 4124 Px (Serotype 1, FVST July 1996, isolated from meat product 1992)
Lm 4127 Px (Serotype 4, FVST July 1996, Isolated from spiced meat roll 1988)
Lm 4140 Px (Serotype 1, bacon, 1992)

General methods used in the imprint experiments

Methods – imprint experiments

Product-to-belt transfer:

- Blocks of ham (10 × 10 cm^2 contact surface by 1.5 cm thick) were spiked with a known number of cells (approx. 5 log CFU/cm^2) on the contact surface
- Spiked ham blocks were gently placed on a pre-cleaned conveyor belt (contact time 20 sec.)
- Bacterial counts (by swabbing) of product before and after imprint as well as bacterial counts on the belt after imprint

Belt-to-product transfer:

- Blocks of ham (10 × 10 cm^2 contact surface by 1.5 cm thick) were spiked with a known number of cells (approx. 5 log CFU/cm^2) on the contact surface
- Spiked blocks were gently placed on a pre-cleaned conveyor belt (contact time 20 sec.)
- A total of 100 non-spiked blocks of ham were then consecutively gently placed on the contaminated area of the belt
- Bacterial counts (by swabbing) of both spiked and non-spiked product before and after imprint, as well as bacterial counts of the belt before and after 100 imprints

General method used in the slicing experiments

Methods – slicing experiments

- Uncontaminated bulk product was sliced
- Bulk product was spiked (contaminated) with a known number of cells
- Spiked bulk product sliced
- Non-spiked bulk product sliced
- Samples extracted from:
  - Bulk product (before and after spiking)
  - Sliced product (spiked and non-spiked)
  - Slicer (selected sampling sites; before, during, and after slicing)
  - Conveyor belt (selected sampling sites; before, during, and after slicing)

Sample handling

Sampling – bulk product

Prior to slicing, surface samples were extracted from spiked as well as non-spiked bulk products.

Using a mould, an area of 100 cm^2 was sampled in order to confirm the spiking level. Two sampling methods were employed: a 1-3 mm depth/surface extraction and swabbing (moist wipe) (Appendix 2c). Three samples of three different

areas were extracted for each sampling method (3 depth/surface, 3 swabs) (Appendix 2d and 2e).

Sampling – sliced product

Samples of sliced product were extracted at predetermined sampling points. Each sample consisted of 1 or 3 slices depending on the amount needed for the bacteriological analysis.

Sampling – environmental

Environmental samples (crumbles, conveyor belt, slicer) were extracted with a sterile gauze wipe wetted with MRD.

When crumbles were extracted, it was ensured that the sampling did not completely remove niches that under normal circumstances would be present.

Before and after slicing of all products, 5-10 swab samples were extracted from the slicer (see the illustration in Appendix 1):

- Table under the slicer blade (only at start-up) (Appendix 1a)
- Belt attachment (only at end) (Appendix 1b)
- Edge of the slicer blade (Appendix 1c)
- Enclosure behind the blade (Appendix 1d)
- Protective shield lower part (Appendix 1e)
- Protective shield upper part (Appendix 1f)
- Rails on feeding chute (Appendix 1g)
- Rim of enclosure (Appendix 1h)
- White board at the end of the feeding chute (Appendix 1i)

Before, during, and after slicing, environmental samples (swabs) were collected from two niches and from the conveyor belt:

- Roller below the conveyor belt (Appendix 1j)
- Table below slicer blade (Appendix 1k)
- Conveyor belt (5 locations) (Appendix 1l)

When sampling the conveyor belt, enough slices (approx. 4-5) were removed to ensure that min. 12 cm of the belt was visible. The belt was swabbed in the entire width (approx. 23 cm) and a length of 10 cm.

Bacteriological analysis

Bacteriological analysis included:

- Total aerobic plate counts (APC) in PCA by seeded pour plate (20°C/5 days)
- *L. monocytogenes* (quantitative) on RAPID'*L.mono* agar by spread plate (37°C/2 days)
- *L. monocytogenes* (qualitative) detected/not detected in 25 g (verification by spread plating on RAPID'*L.mono* agar) (37°C/2 days)
- *L. monocytogenes* (semi-quantitative) cf. analysis protocols:
  - "Semikvantitativ bestemmelse af *Listeria monocytogenes* i fødevarer"
  - "Semikvantitativ bestemmelse af *Listeria monocytogenes* i miljøprøver (svaber)"

Results & Discussion

Basic data

Basic data

During all the experiments, various basic data was collected to document the experimental conditions and prerequisites. These are summarised in this section.

Temperature & humidity in the room

Temperature and relative humidity were measured with two Testo loggers placed below the slicer and below the chute feeding the bulk product and logged every 10 minutes. The temperature in the room was approx. 7-8°C and the rel. humidity approx. 60-70% (Appendix 3).

Bacterial counts in cultures

The Listeria counts (*Lm*) of the spiking cultures and cocktails were determined on RAPID'*L.mono* (37°C/2 days). The individual cultures had (with two exceptions) counts of 9.0-9.6 log CFU/ml as anticipated. For the spiking cocktails, the counts were in the planned ranges, with minor deviations in the two cases where one of the individual cultures had not grown to the expected numbers. To rule out contamination, aerobic plate counts (APC) were determined on PCA (20°C/5 days). The difference between *Lm* counts and APC were generally < 0.2 log CFU/ml, which is acceptable. The difference was slightly higher (0.4 log CFU/ml) for the UL experiment, but as the *Lm* counts were higher than the APC, it did not give rise to concern regarding contamination (Appendix 4).

Bacterial counts – non-spiked bulk product

Bacterial counts (APC and/or *Lm*) of the surface of non-spiked bulk products were determined by swabbing (n=3) and by the depth/surface method (n=3) cf. the section '*Sampling – bulk product*'. Results are listed in Appendix 5. In all but one experiment, the bacterial counts on the non-spiked product were negligible. In one experiment (Ham L (1)), there seemed to be either a bacterial flora on the hams or a contamination of the sample. This did not give cause for concern regarding influencing the results of the experiment, since at the same time no Listeria could be detected.

Bacterial counts – spiked bulk product

Bacterial counts (APC and/or *Lm*) of the surface of spiked bulk products were determined by swabbing (n=3) and by the depth/surface method (n=3) cf. the section '*Sampling – bulk product*'. Results are listed in Appendix 6. The bacterial counts were in line with the calculated contamination levels of 5-6 log CFU/cm^2 for the medium level, 3-4 log CFU/cm^2 for the low-level, and 0.1-1 log CFU/cm^2 for the ultra-low-level spiked products. The two sampling methods ('depth/surface' and 'swab') yielded similar counts.

Bacterial counts on sliced product

In selected slicing experiments, the bacterial counts (APC and *Lm*) were determined both for slices of non-spiked bulk product (run prior to running the spiked bulk product through the slicer) and for slices of the spiked bulk product. Results are listed in Appendix 7.

The data showed that there was a slightly lower level of *Lm* on the spiked slices than on the spiked bulk product, thus reflecting that some of the bacteria were transferred to the equipment. This was not the case for the APC counts, which may be due to improper cleaning of the equipment and that bacteria trapped in

insufficiently cleaned niches of the slicer were 'flushed out' onto the first clean product being sliced.

2019 experiments

2019 experiments

In the first year of the project, three experiments were conducted. The imprint experiment explored the transfer rates between product and conveyor belt through direct imprint, and the two slicing experiments dived further into this product-to-belt transfer using an industrial slicer. The slicing experiments also looked at the effects of interval cleaning.

Bacterial transfer between product and conveyor belt

The imprint experiment yielded the following results:

Transfer from spiked bulk product to conveyor belt

- 5.5 log CFU/cm^2 (product) ⇨ 3.8 log CFU/cm^2 (belt)

Transfer from contaminated conveyor belt back to clean product

- Ham #30: 0.2 log CFU/cm^2
- Ham #60: < 0.5 log CFU/cm^2
- Ham #90: < 0.5 log CFU/cm^2/detected

Residual contamination on belt after the imprint of 100 blocks of ham

- 1.6 log CFU/cm^2

Calculated transfer rate (product-to-belt): approx. 2%

The initial slicing experiments ('Ham M (1)' and 'Ham M (2)') yielded the following results:

Transfer from spiked bulk product to conveyor belt

- 5.5 log CFU/cm^2 ham ⇨ 3 log CFU/cm^2 belt

Calculated transfer rate (product-to-belt): approx. 0.3%

Table 1. *Lm* count after slicing clean product (log CFU/cm^2) with interval cleaning (IC) and without interval cleaning (NIC)

Sampling point		IC	NIC	Transfer rate
Location	Ham no.	log CFU/cm^2	log CFU/cm^2	Belt-to-product
Product	10	0	1.7 to 2.8	Approx. 1-10%
Belt	10	-0.6 to -1	0.7 to 1.8	
Product	15	0	1.0 to 2.5	Approx. 1-20%
Belt	15	0 to -1	0.3 to 1.7	

Note: 0 log CFU/cm^2 = 1 CFU/cm^2

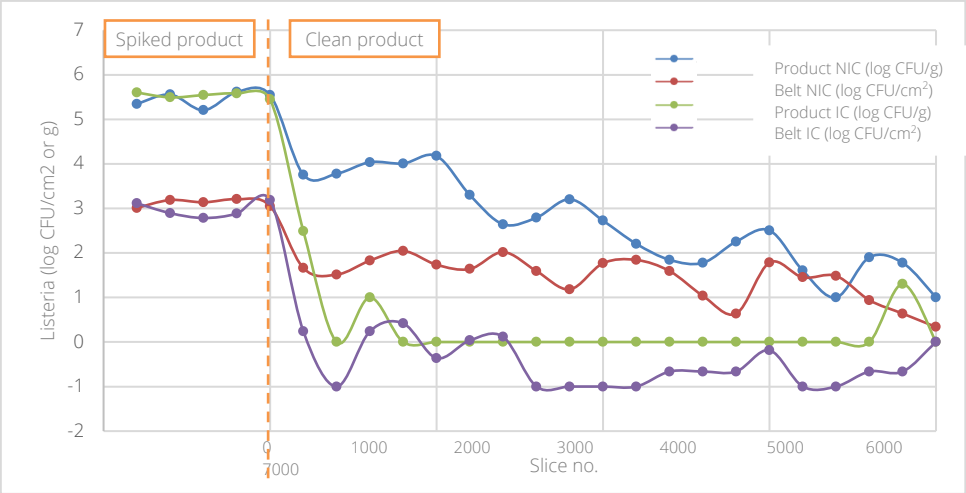


Figure 1. *Lm* counts on product and belt. Up until the dotted line, spiked product was run through the slicer. At the point marked by the dotted line, the slicer (but not the belt) was either thoroughly wiped down with alcohol wipes (interval cleaning = IC) or not wiped down (no interval cleaning = NIC), after which clean product was sliced (a total of 15 sticks of ham = approx. 7000 slices). The analysed slices were collected immediately after slicing without contact to the conveyer belt (clean plastic bags were placed on the conveyer belt under the sampled slices).

The effect of interval cleaning is significant, as can be seen by the drastic drops in *Lm* on both product and belt (green and purple curves) compared to the moderate drops when proceeding to slice product without interval cleaning (blue and red curves).

It is noteworthy that *Lm* was found in all samples.

More results regarding product to belt transfer are discussed in the section '*Bacterial transfer – product to niches*'.

2020 experiments

2020 experiments

To further investigate the transfer of *Lm* between product and equipment, four more slicing experiments were conducted in 2020. Two of these (Ham L (1) and Baloney L) were conducted on the same slicer as the initial (2019) experiments and two (Ham L (2) and Ham UL) were conducted on a slicer of a different model. The findings are summarised in the following sections.

Bacterial transfer – product to equipment

The slicer was swabbed in multiple locations (Appendix 1) before it was contaminated with the spiked bulk product and again at the very end of the experiment. The difference between the before and after numbers reflects how many bacterial cells that have been trapped in the various locations of the equipment. Results are listed in Appendix 9 and illustrated in Figure 2.

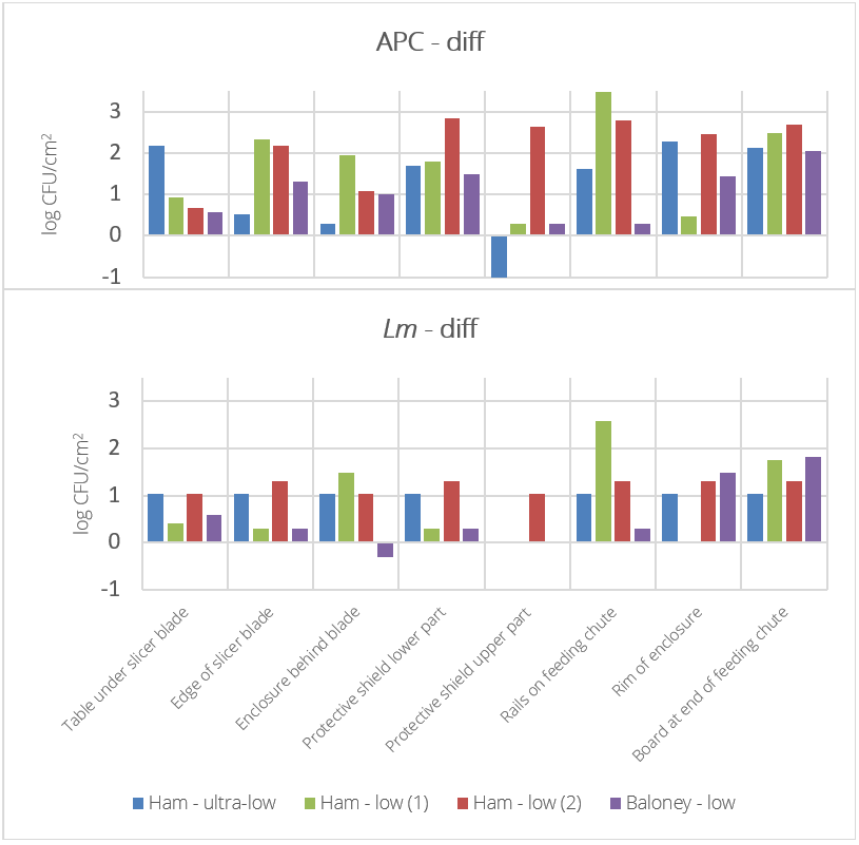


Figure 2. Difference (from before contamination to after the end of the experiment) between contamination levels of equipment (ultra-low = spiking level 1-10 CFU/g; low = spiking level 1,000-10,000 CFU/g).

The results indicate:

- Listeria accumulates on almost all the sampled areas, although somewhat inconsistently. Interpretations are cautious, as data is based on one sampling before and one after, for each location, and because it is difficult to standardize sampling of irregular areas like these.
- None of the experimental setups generated results that were significantly different than the rest.
- The generally higher APC counts for ham (vs. baloney) could be explained by low-level contamination of the bulk product which attaches to the equipment by direct contact. Since the ham is square, it will have a larger contact surface than the round baloney.
- The table under the slicer blade most likely would accumulate more than what the numbers show, but removal of crumbles for sampling halfway through the experiment probably has skewed the results. For comparison, crumbles gathered on the board at the end of the feeding chute in much the same way as on the table under the slicer were not removed for sampling during the experiment and therefore gave a perhaps more realistic accumulation result.
- Even after slicing more than 10,000 slices of 'clean' bulk product, *Lm* could still be found in several places on the equipment.

Bacterial transfer – product to niches

Samples were extracted from two different niches (table under the slicer blade and roller under the conveyor belt) as well as from the conveyor belt (under slices, average of five locations) at selected times during the slicing process, to monitor the development of bacterial transfer from product to niches and belt. The niches and belt were swabbed 1) pre-contamination ('Clean'), 2) immediately after the contamination incidence ('Cont'), 3) post-contamination (after approx. 4,500 slices), and 4) post-contamination (after approx. 10,600 slices). Results are listed in Appendix 8 and illustrated in Figures 3 a-d.

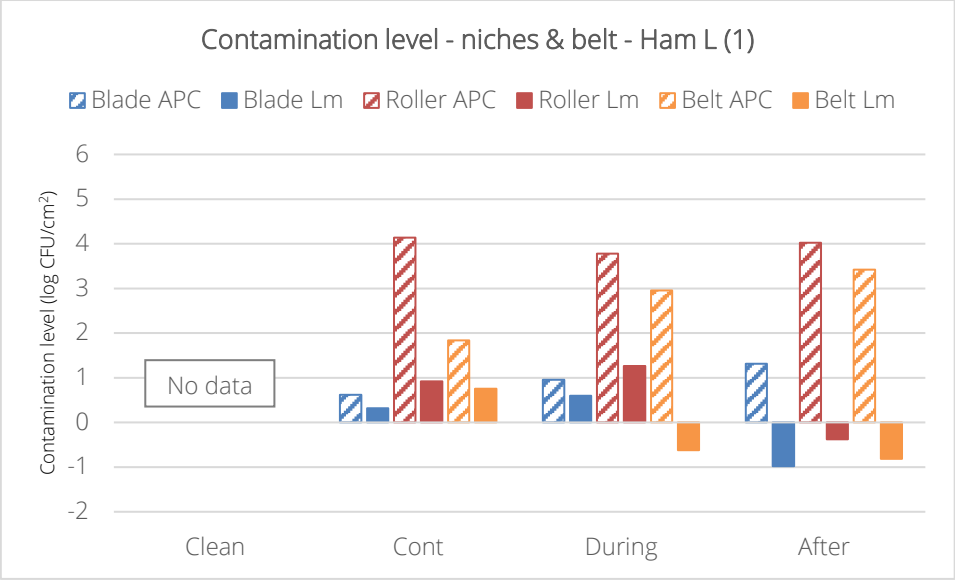


Figure 3a. Contamination levels in two niches and on the conveyor belt during 1st ham experiment with low-level contamination of the bulk product. No data available for 'Clean' due to an error during the sampling process.

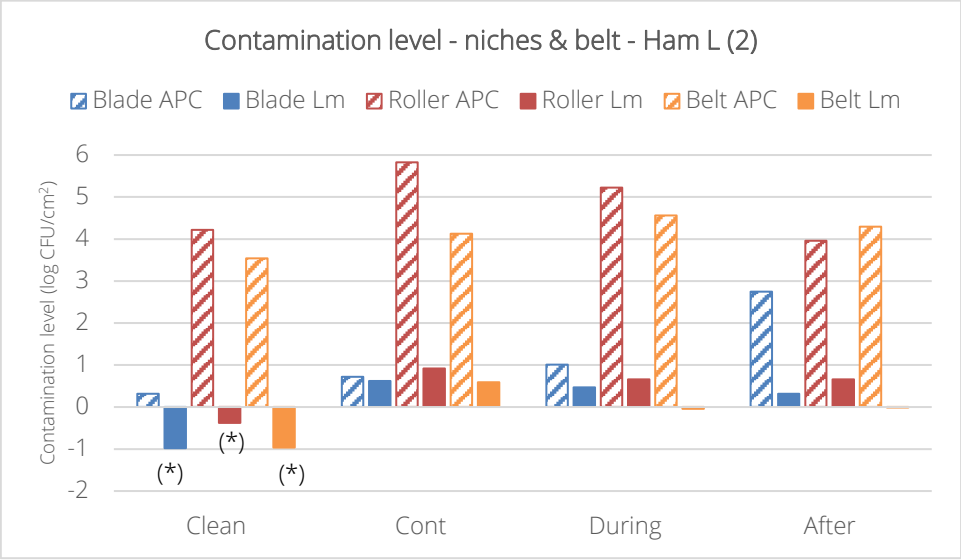


Figure 3b. Contamination levels in two niches and on the conveyor belt during 2nd ham experiment with low-level contamination of the bulk product. (*) = not detected (bars indicate detection limit).

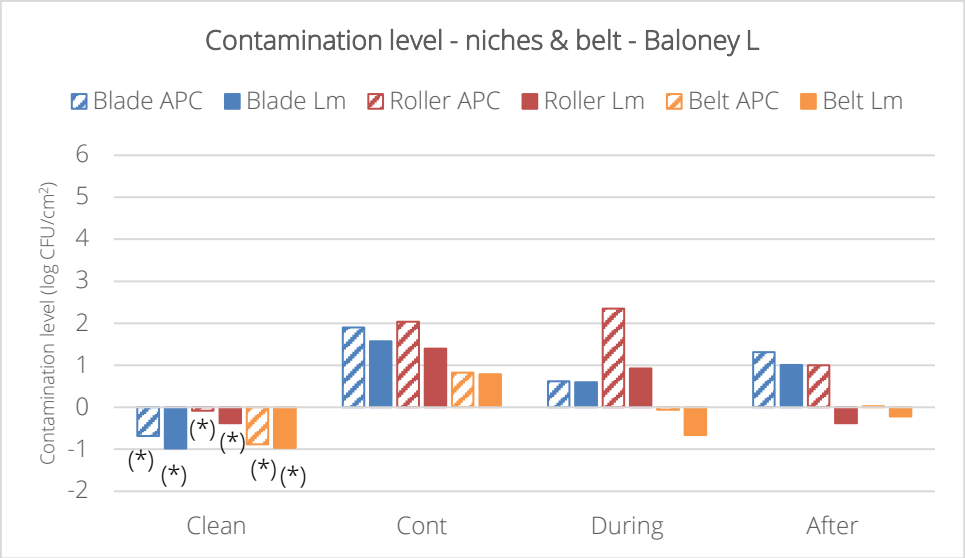


Figure 3c. Contamination levels in two niches and on the conveyor belt during baloney experiment with low-level contamination of the bulk product. (*) = not detected (bars indicate detection limit).

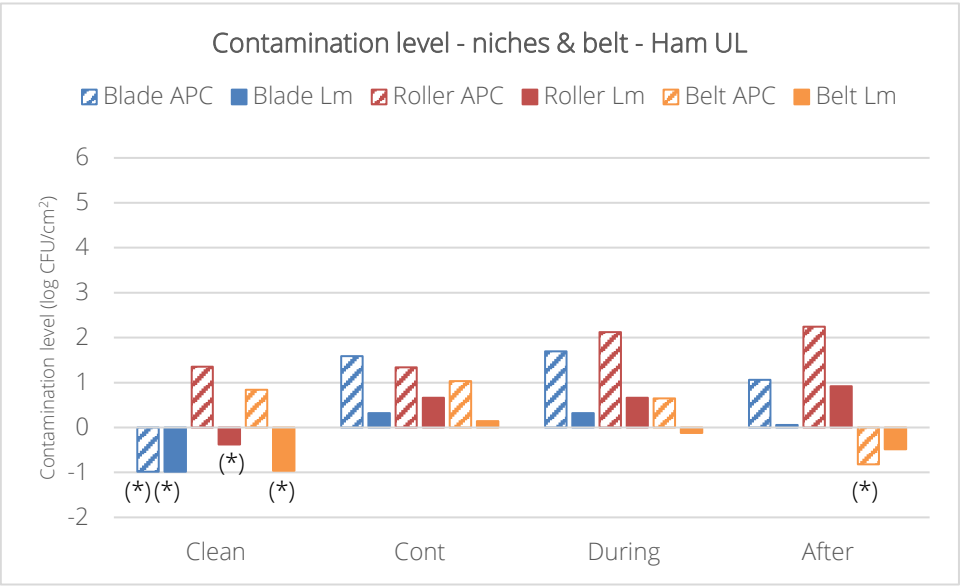


Figure 3d. Contamination levels in two niches and on the conveyor belt during ham experiment with ultra-low-level contamination of the bulk product. (*) = not detected (bars indicate detection limit).

- The *Lm* results (Figures 3 a-d) illustrate that a one-time contamination incident leads to an immediate accumulation in the equipment, which slowly tapers off but never totally disappears.
- The APC results demonstrate that a continual feed of contaminants from low-level contaminated bulk product or an initial low-level contamination due to insufficient cleaning leads to an accumulation (or at least steady-state situation) of bacterial cells in the equipment. The large difference between APC and *Lm* counts in the ham experiments indicates presence of an initial bacterial flora on the bulk product (Appendix 5) or in the equipment due to improper cleaning (data not available).

- The results for the baloney (Figure 3c) show a more consistent relation between APC and *Lm* counts (vs. the ham results), indicating a low initial bacterial flora from bulk product and equipment.

Bacterial transfer – product to equipment – theoretical calculations

To get an indication (best case/worst case) of how many *Lm* cells that stay on the product, and how many that 'escape' into the environment, some theoretical calculations were made (Table 2). Columns A-C show the inoculation level on the bulk product transformed into the calculated cell count on three slices (the log CFU count per cm^2 recalculated to the CFU count per cm^2 followed by the recalculation into CFU per gram (using the average area/weight of the slices)). Column D is the measured count on three slices (InvLog of the counts listed in Appendix 6). The difference between the calculated count (i.e., how many cells would be on the slices if all the inoculated cells stayed on the product during slicing) and the measured count (actual number of cells on three slices) would theoretically indicate how many cells that have 'escaped' (i.e., how many cells have been transferred to the equipment – column E and F).

Table 2. Calculations for *Lm* cells transferred from contaminated bulk product to equipment. Difference = theoretical "escaped" *Lm* to the equipment.

	A	B	C	D	E	F
	Log CFU/cm^2 (bulk)[a)]	CFU/cm^2 (bulk)	CFU/g (bulk)[b)]	CFU/g (slices)[c)]	Difference (CFU/g)	Difference (%)
Ham L (1) (wc)	3.42 (3.4 + 0.02)	2630	1035	794	241	23.2
Ham L (1) (bc)	3.38 (3.4 – 0.02)	2399	944	794	149	15.8
Ham L (2) (wc)	3.21 (3.1 + 0.11)	1622	638	395	243	38.0
Ham L (2) (bc)	2.99 (3.1 – 0.11)	977	384	395	-11	-2.8
Baloney L (wc)	3.93 (3.8 + 0.13)	8511	5852	3981	1871	32.0
Baloney L (bc)	3.67 (3.8 – 0.13)	4677	3216	3981	-765	-23.8
Ham UL (wc)	1.00 (1.0 + 0.00)	10	4	1	3	69.2
Ham UL (bc)	1.00 (1.0 – 0.00)	10	4	1	3	69.2

a) Depth/surface *Lm* count ± std.dev. (derived from Appendix 6)
b) Derived from edge area and weight calculations for three slices of product (data not shown)
c) InvLog results from Appendix 7
wc = worst case
bc = best case

The results (Table 2) illustrate that the inherent uncertainty of measurement connected to this type of data prevents any firm conclusions. The calculations for Ham L (1) (with a standard deviation of 2%) indicate that approx. 15-25% of the *Lm* cells are transferred to the equipment and other contact surfaces. Due to the rather large standard deviations in the Ham L (2) and Baloney L results, the calculations are a bit more difficult to interpret, as the negative numbers in the 'best case' scenario would indicate that *Lm* cells are transferred from the equipment to the spiked product, which is highly unlikely. Were the calculations for all experiments done with a standard deviation equal to the one for Ham L (1) (i.e., 2%), the transfer from spiked product to equipment would be approx. 68-71% for Ham UL, 16-23% for Ham L (1), 18-24% for Ham L (2), and 4-12% for Baloney L (calculations not shown).

Bacterial transfer – equipment to product

Product samples (slices) were extracted at regular intervals during the slicing period to monitor the transfer of Listeria from equipment to product. The Listeria contamination was enumerated either directly (quantitative method on RAPID'*L.mono*) or indirectly by a semi-quantitative method. The semi-quantitative method was used when numbers were below the detection limit for the direct quantitative method. Results from the semi-quantitative method will indicate whether counts are within a 1 log interval (e.g., 0.04-0.4 log CFU/g). This is illustrated by a vertical line (1 log long) in Figures 4 a-c.

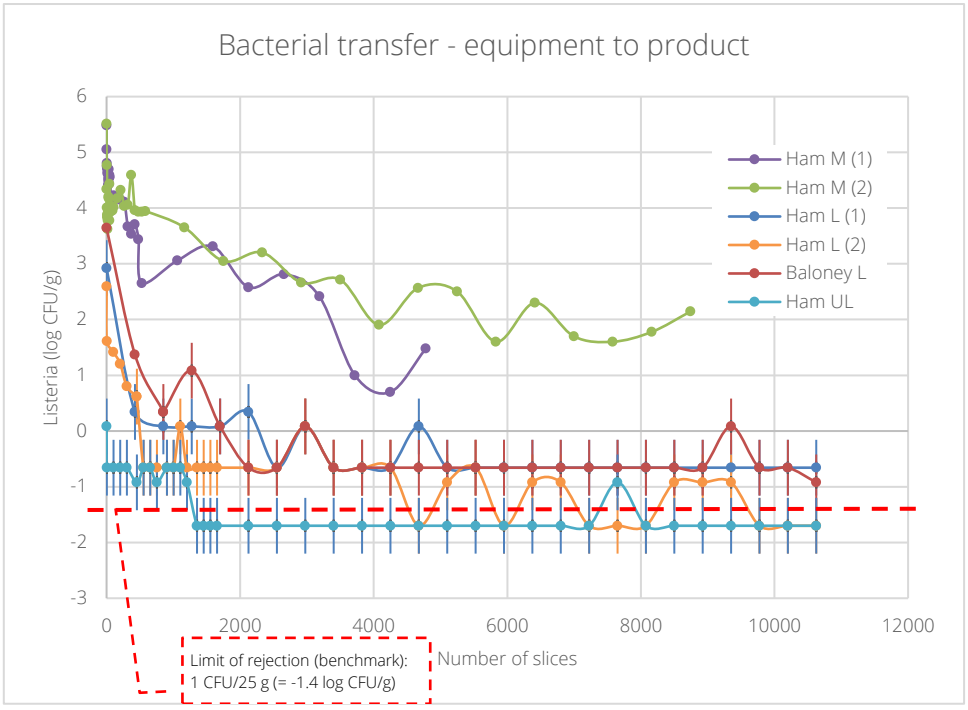


Figure 4a. Listeria counts (log CFU/g) on sliced product.

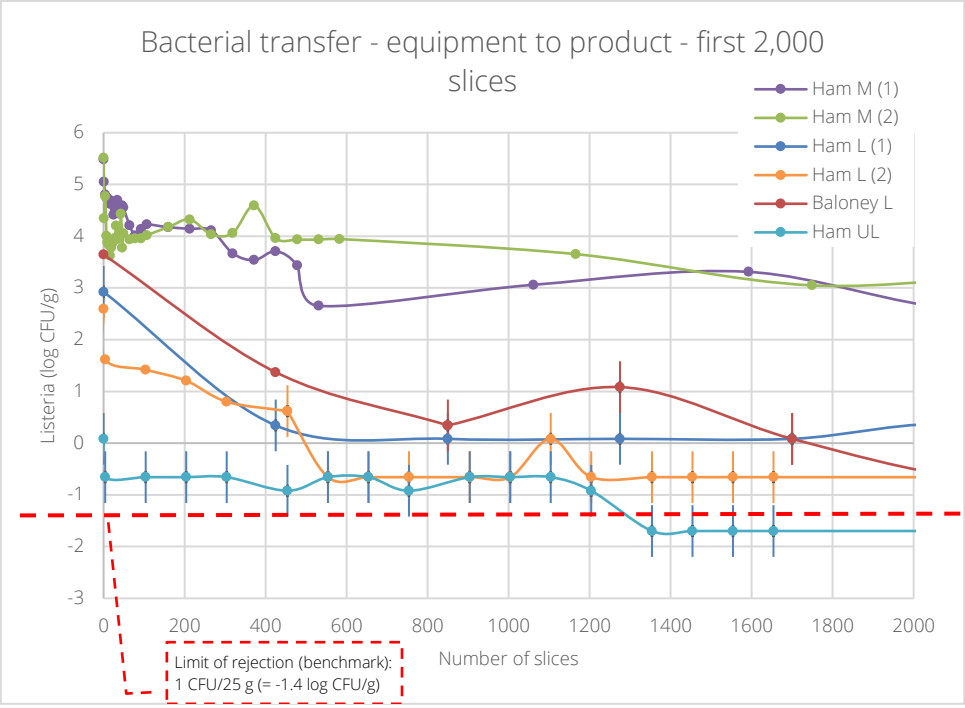


Figure 4b. Listeria counts (log CFU/g) on sliced product. A close-up look at the first 2,000 slices.

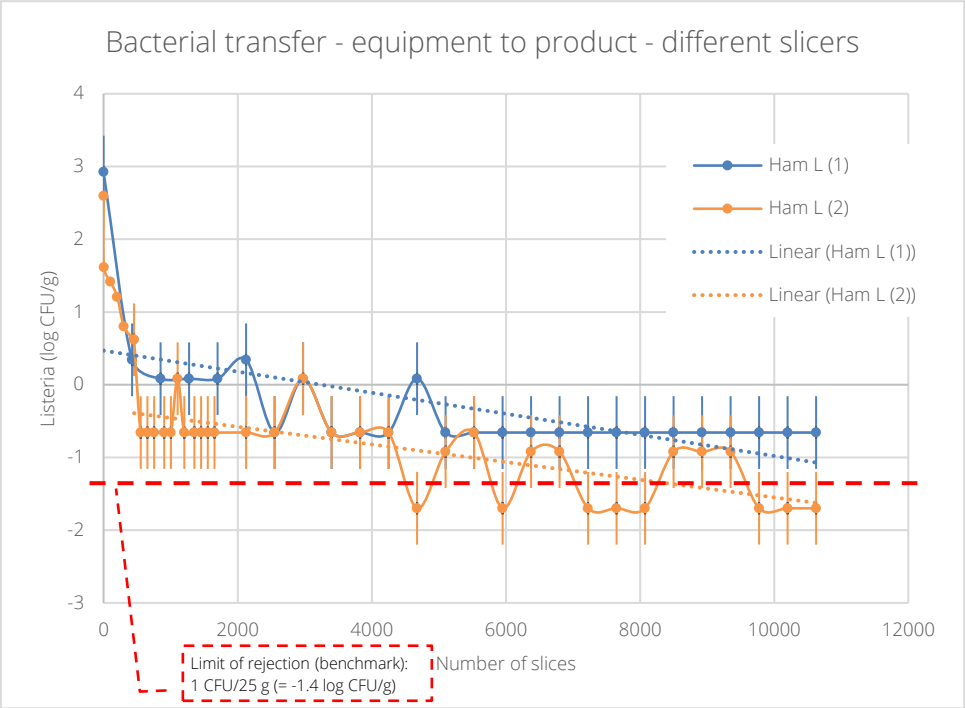


Figure 4c. Listeria counts (log CFU/g) on sliced product for two experiments with the same product and initial contamination level but conducted on two different slicers.

- The first point on each curve (Slice 0) is the *Lm* count from the sliced spiked product and is defined as the starting point.
- The subsequent data points are from 'clean' product, sliced after the contamination event.
- In the two experiments where the initial contamination level was relatively high (5-6 log CFU/g) there was a 4-log drop within the first 4-6,000 slices. Although these two experiments gave first indications of what to expect, they also revealed the problems with choosing such a high level of contamination: 1) it is far from realistic that such a severe contamination should occur in real life and 2) it would be impossible to reach a point where *Lm* can no longer be detected within a reasonable timeframe (i.e. number of slices).
- In all four low and ultra-low contamination experiments most of the contamination was transferred from equipment to product within 2,000 slices, to reach a relative equilibrium after that.
- The ultra-low experiment best reflects a real-life contamination event, and it is noteworthy that after approximately 1,300 slices, the contamination level drops below the rejection limit (benchmark) of 1 CFU in 25 g (Figures 4a and 4b) and remains there, except for one sample (slice no. 7,650) (Figure 4a). This 'late occurrence' may very well be caused by an occasional 'release' from one of the niches, e.g., the roller underneath the belt. The roller trapped significant amounts of slicing 'crumbles' (Appendix 1j), and occasionally some of this would be released to the belt and subsequently stick to a clean slice.
- Results for ham sliced on two different slicers were slightly different (approximately 0.7 log difference between trendlines) (Figure 4c). This may be caused by a combination of two circumstances: 1) the initial count (on spiked bulk product) was 0.3-0.4 log higher in Ham L (1) than Ham L (2) (Appendix 6) and 2) it appears that more *Lm* were trapped in niches in Ham L (2) than in Ham L (1) (Figures 3a and 3b). The first circumstance is independent of the equipment, but the second circumstance may be rooted in small differences in the equipment design.
- Listeria was detected in all samples although at extremely low levels after approx. 1,300 samples in a close to realistic situation.

Mathematical modelling

Fitting data to a mathematical model

Mathematical modelling was applied to the data generated in the six slicing experiments. First, it was investigated whether different datasets differed significantly from a statistical point of view.

Data was fitted to a double-logarithmic model: Ln(Log CFU) = a + b × slice number

Analysis of the significance of the variables 'product', 'slicer model', and 'repetition' could be carried out as an analysis of covariance of Ln(Log CFU):

$$\text{Ln(Log CFU)} = a + a_{experiment} + b_{experiment} \times \text{slice number}$$

and included a test of four different hypothesis using Student's T-test ($a_{experiment}$ = 0 and $b_{experiment}$ = 0)[1].

The estimated parameters a and b are used to predict 'Log CFU', i.e.,

$$\text{Predicted Log CFU} = \text{Exp}(a + b \times \text{slice number})$$

Residuals for each slice number were determined as:

$$\text{Res(slice number)} = \text{Predicted Log CFU} - \text{Log CFU},$$

while assuming that the residuals are following a normal distribution and that the normal distribution is independent of the slice number.

The standard deviations of the residuals is the mean deviation used to calculate the probability of finding more than 1 CFU/g or more than 1 CFU/25 g (benchmark value), respectively, on a slice following a contamination incident was estimated for up to 1,000,000 slices.

Results of statistical analysis:

Table 3. Student's T-test of four different hypothesis: H1 (two repetitions at medium spiking level), H2 (two different slicers), H3 (two different products), H4 (two different products sliced on each their slicer). In all four cases $a_{experiment}$ = 0. '$b_1 - b_2$' denotes the estimated difference between the slopes of the two fitted models with corresponding 95% confidence interval. 'Significance level' denotes the p-level for the T-test (*** = very significant, NS = not significant).

Hypothesis	Spiking level	'$b_1 - b_2$'	Confidence interval	Significance level
H1: Ham M(1) ≠ Ham M(2)	Log CFU ≈ 5.5 CFU ≈ 300,000	0.0002	± 0.000043	***
H2: Ham L(1) ≠ Ham L(2)	Log CFU ≈ 2.7 CFU ≈ 500	0.00012	± 0.000059	***
H3: Ham L(2) ≠ Baloney L	Log CFU ≈ 3.3 CFU ≈ 2,000	0.000018	± 0.000045	NS
H4: Ham L(1) ≠ Baloney L	Log CFU ≈ 3.1 CFU ≈ 1,250	0.0001069	± 0.000061	***

According to hypothesis 1 (H1), the two runs at medium level are significantly different, although they were conducted on the same slicer, with the same product and at almost exactly same initial contamination level (5.5 and 5.6 CFU/cm^2). But the fact that they were very different in length (see Figure 4a) may have triggered the statistical difference. Thus, in spite of the result of the T-test, these two runs are considered equal and as two repetitions of the same experiment.

[1] Observe, if the Log CFU value was a negative number, it was temporarily transformed to a positive value during the calculations. The transformation did not affect the conclusion of the statistical evaluation.

Hypothesis 2 (H2) tests whether two runs of the same product with the same contamination level but on two different slicers are different. The T-test shows a significant difference, which would indicate that it might matter, which slicer is used.

Hypothesis 3 (H3) looks at comparing two different products with the same contamination level, and sliced on the same slicer. The T-test is not significant, thus inducating that the type of product is irrelevant.

Hypothsis 4 (H4) compares two different products with the same contamination level, but sliced on two different slicers. The T-test shows a significant difference, which (compared to H2 og H3) must be attributed to the difference in slicers, rather than the difference in product.

By combining the datasets for Ham M(1) and Ham M(2), a dataset reflecting the natural variation at the specific contamination level was obtained. Similary, the datasets for Ham (L2) and Baloney L were merged. Models were fitted to each of the two merged datasets, and a model was fitted to the Ham UL dataset, thus creating a model for each contamination level (ultra-low, low, and medium). For each model, a table was created (Tables 4-6), listing the probability of finding more than one *Lm* in 1 gram (or 25 g) in a slice of 'clean' product after a contamination incident. These tables may be used to get a very causious indication of how long and to which extend a contamination could be expected to 'linger on' in case of an accidental contamination incident.

Table 4. Statistical modelling of predicted contamination level and probability (in %) of finding *Lm* in subsequent slices in case of a contamination incident at ultra-low level (0.1-1 log CFU/g).

	Predicted contamination		Probability of finding	
Slice number	Log CFU/g	CFU/g	>1 CFU/g	>1 CFU/25 g
100	-1.18	0.07	0%	74%
1,000	-1.27	0.05	0%	65%
10,000	-1.78	0.02	0%	13%
100,000	-2.00	0.01	0%	4%
1,000,000	-2.00	0.01	0%	4%

Table 5. Statistical modelling of predicted contamination level and probability (in %) of finding *Lm* in subsequent slices in case of a contamination incident at low level (3-4 log CFU/g).

	Predicted contamination		Probability of finding	
Slice number	Log CFU/g	CFU/g	>1 CFU/g	>1 CFU/25 g
100	0.26	1.82	78%	100%
1,000	0.12	1.33	64%	100%
10,000	-0.87	0.13	0%	94%
100,000	-2.00	0.01	0%	4%
1,000,000	-2.00	0.01	0%	4%

Table 6. Statistical modelling of predicted contamination level and probability (in %) of finding *Lm* in subsequent slices in case of a contamination incident at medium level (5-6 log CFU/g).

	Predicted contamination		Probability of finding:	
Slice number	Log CFU/g	CFU/g	>1 CFU/g	>1 CFU/25 g
100	4.26	18117	100%	100%
1,000	3.73	5342	100%	100%
10,000	0.99	10	99%	100%
100,000	0.00	1	50%	100%
1,000,000	0.00	1	50%	100%

Tables 4-6 may support the risk assessment after a contamination incident, but due to the limited data and keeping in mind that *Lm* may very well be accumulating in niches in the equipment, the tables should be used with much caution.

Conclusion

Conclusion

When the method of direct imprint was used, the transfer rate of *Listeria monocytogenes* (*Lm*) between ham and conveyor belt (product-to-belt-to-product) was approx. 2%. However, on a pre-contaminated conveyer belt, *Lm* could still be detected on the belt after 100 imprints with "clean ham".

Interval cleaning has a significant effect on the levels of *Lm* on both product and conveyor belt and may be considered a good tool to knock down accidental contaminations during shifts.

Slicing on two different slicers gave varying results. This may be partly due to the slight difference in initial contamination level and partly due to small differences in the equipment design.

A one-time contamination incident led to an immediate accumulation in the equipment, which slowly tapered off but never totally disappeared. Even after slicing more than 10,000 slices of 'clean' bulk product, *Lm* could still be found in several places (niches) on the equipment. Niches harbouring *Lm* are problematic, as they may be a continuous (although sporadic) source of contamination for a subsequent clean product.

After a low or ultra-low contamination incident, most of the contamination was transferred from the equipment to product within 2,000 slices, to reach a relative equilibrium after that. For the ultra-low contamination incident, which best mimics a 'real life' situation, it is noteworthy that after approximately 1,300 slices, the contamination level dropped below the rejection limit of 1 CFU in 25 g. But due to sporadic release from niches, it cannot be ruled out that occasional low-level counts of *Lm* can occur on later slices, unless these niches are cleaned.

Tables were generated, listing the probability of finding more than one *Lm* in 1 gram (or 25 g) in a slice of 'clean' product after a contamination incident. The tables could aid in a dynamic risk assessment and ultimately minimize the waste of

product after a confirmed contamination incident. Furthermore, they may provide valuable input to the dialogue with food inspection authorities following an incident.

GENERAL REQUIREMENTS OF FOOD LAW

Article 14

Food safety requirements

1. Food shall not be placed on the market if it is unsafe.

2. Food shall be deemed to be unsafe if it is considered to be:

(a) injurious to health;

(b) unfit for human consumption.

3. In determining whether any food is unsafe, regard shall be had:

(a) to the normal conditions of use of the food by the consumer and at each stage of production, processing and distribution, and

(b) to the information provided to the consumer, including information on the label, or other information generally available to the consumer concerning the avoidance of specific adverse health effects from a particular food or category of foods.

4. In determining whether any food is injurious to health, regard shall be had:

(a) not only to the probable immediate and/or short-term and/or long-term effects of that food on the health of a person consuming it, but also on subsequent generations;

(b) to the probable cumulative toxic effects;

(c) to the particular health sensitivities of a specific category of consumers where the food is intended for that category of consumers.

5. In determining whether any food is unfit for human consumption, regard shall be had to whether the food is unacceptable for human consumption according to its intended use, for reasons of contamination, whether by extraneous matter or otherwise, or through putrefaction, deterioration or decay.

6. Where any food which is unsafe is part of a batch, lot or consignment of food of the same class or description, it shall be presumed that all the food in that batch, lot or consignment is also unsafe, unless following a detailed assessment there is no evidence that the rest of the batch, lot or consignment is unsafe.

7. Food that complies with specific Community provisions governing food safety shall be deemed to be safe insofar as the aspects covered by the specific Community provisions are concerned.

8. Conformity of a food with specific provisions applicable to that food shall not bar the competent authorities from taking appropriate measures to impose restrictions on it being placed on the market or to require its withdrawal from the market where there are reasons to suspect that, despite such conformity, the food is unsafe.

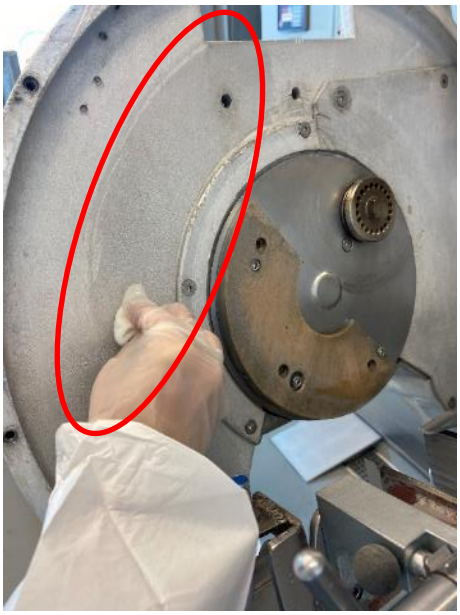
9. Where there are no specific Community provisions, food shall be deemed to be safe when it conforms to the specific provisions of national food law of the Member State in whose territory the food is marketed, such provisions being drawn up and applied without prejudice to the Treaty, in particular Articles 28 and 30 thereof.

Sampling in the slicer

1a. Table under the slicer blade

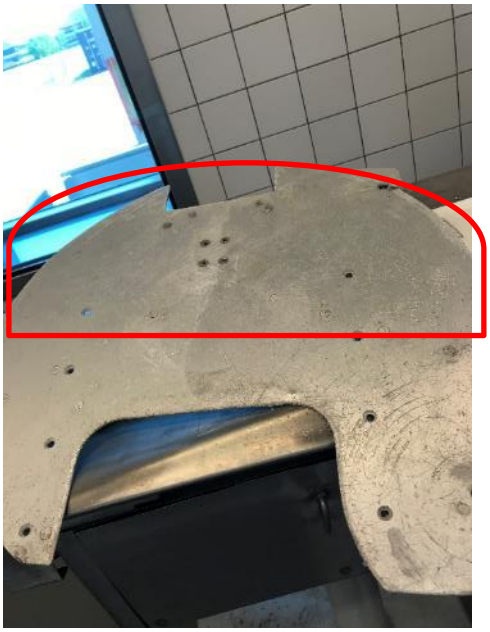
1b. Belt attachment

1c. Edge of the slicer blade



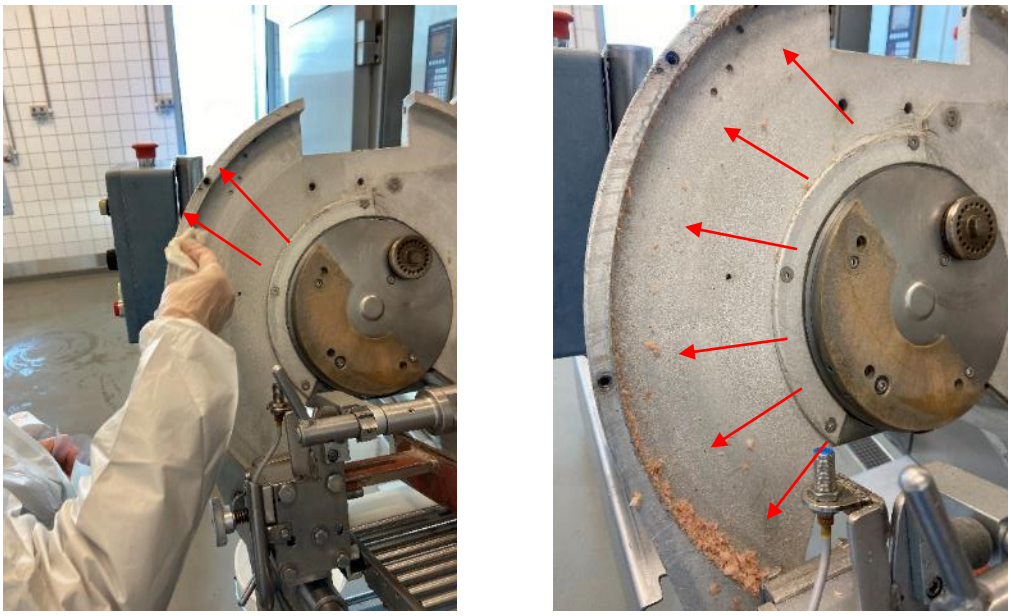
1d. Enclosure behind the blade

1e. Protective shield lower part

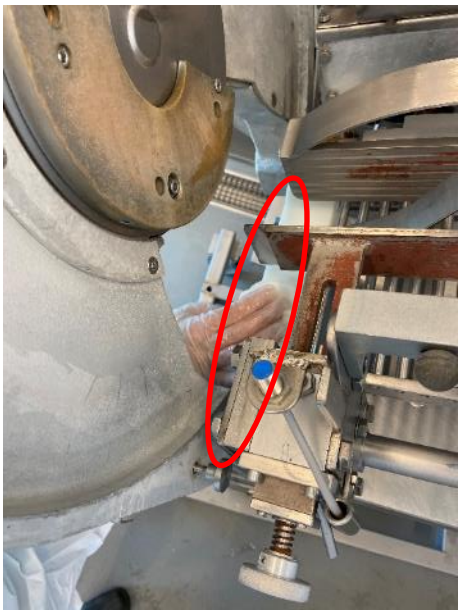


1f. Protective shield upper part

1g. Rails on feeding chute



1h. Rim of enclosure



1i. White board at the end of the feeding chute

1j. Roller below the conveyor belt

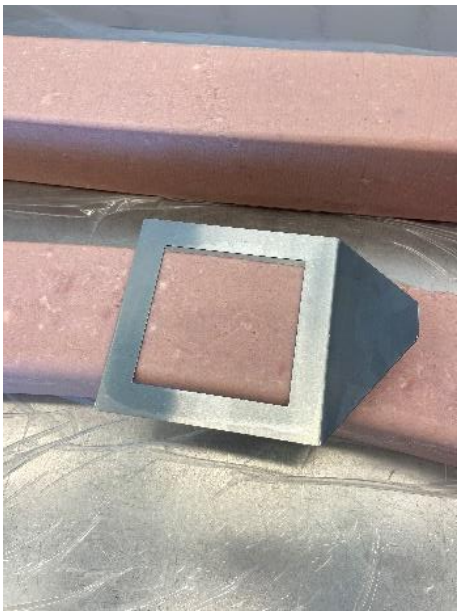
1k. Table below the slicer blade

1l. Conveyor belt

Miscellaneous illustrations

2a. Hams are lined up

2b. Spiking the bulk product



2c. Mould for swab and depth/surface sampling

2d. Swabbing the bulk product

2e. Depth/surface sampling of bulk product

2f. Slicing in progress…

2g. End piece

2h. Coating on the slicer blade

Temperature and rel. humidity during the slicing experiments

The temperature and the relative humidity were measured with two Testo loggers placed below the slicer and below the chute feeding the bulk product and logged every 10 minutes. The temperature in the room was approx. 7-8°C and the rel. humidity approx. 60-70%.

2019 experiments

Ham M (1)	Logger 1 (n=54)		Logger 2 (n=53)	
	average	std.dev.	average	std.dev.
Temperature (°C)	7.3	0.5	6.4	0.6
Rel. humidity (% RH)	58.8	2.5	62.1	3.1
Ham M (2)	**Logger 1** (n=28)		**Logger 2** (n=25)	
	average	std.dev.	average	std.dev.
Temperature (°C)	7.27	0.44	8.26	67.96
Rel. humidity (% RH)	66.10	2.21	0.31	3.55

2020 experiments

Ham UL	Logger 1 (n=54)		Logger 2 (n=53)	
	average	std.dev.	average	std.dev.
Temperature (°C)	10.2	0.48	10.1	0.47
Rel. humidity (% RH)	79.1	5.78	88.1	6.61
Ham L (1)	**Logger 1** (n=28)		**Logger 2** (n=25)	
	average	std.dev.	average	std.dev.
Temperature (°C)	8.2	2.09	7.6	2.31
Rel. humidity (% RH)	55.3	8.34	58.6	9.21
Ham L (2)	**Logger 1** (n=25)		**Logger 2** (n=50)	
	average	std.dev.	average	std.dev.
Temperature (°C)	5.5	1.08	5.6	1.03
Rel. humidity (% RH)	68.0	9.66	75.4	11.66
Baloney L	**Logger 1** (n=25)		**Logger 2** (n=25)	
	average	std.dev.	average	std.dev.
Temperature (°C)	8.3	1.44	7.4	1.61
Rel. humidity (% RH)	61.1	6.46	63.8	7.38

Bacterial counts in spiking cultures and cocktail

The Listeria counts (*Lm*) in the spiking cultures and cocktail were determined on RAPID'*L.mono* (37°C/2 days). As a test for contamination, aerobic plate counts (APC) were determined on PCA (20°C/5 days) (APC not analysed for 'Imprint' and 'Ham M (2)' experiment).

2019 experiments

	Imprint		Ham M (1)		Ham M (2)	
	Lm	APC	*Lm*	APC	*Lm*	APC
LM 3012	9.3	N/A	9.3	9.1	9.2	N/A
LM 4106	9.3	N/A	9.0	9.1	9.2	N/A
LM 4124	9.2	N/A	9.2	9.3	9.3	N/A
LM 4127	8.6	N/A	9.3	9.4	9.3	N/A
LM 4140	9.3	N/A	9.2	9.3	9.2	N/A
Spiking cocktail	9.3	N/A	8.2	8.2	8.2	N/A

2020 experiments

	Ham UL		Ham L (1)		Ham L (2)		Baloney	
	Lm	APC	*Lm*	APC	*Lm*	APC	*Lm*	APC
LM 3012	9.3	9.1	9.1	9.2	8.3	8.5	9.2	9.3
LM 4106	9.3	9.4	9.1	9.3	9.5	9.3	9.3	9.3
LM 4124	9.3	9.2	9.2	9.3	9.4	9.3	9.3	9.3
LM 4127	9.3	9.1	9.3	9.3	9.6	9.3	9.2	9.4
LM 4140	9.4	9.3	9.2	9.3	9.4	9.4	9.2	9.4
Spiking cocktail	3.4	3.0	5.5	5.6	5.5	5.4	6.3	6.5

APC + Listeria counts (CFU/cm²) for the surface of non-spiked bulk product

2019 experiments (depth/surface sampling method)

n=3	Imprint	Ham M NIC (1)	Ham M IC (1)	Ham M NIC (2)	Ham M IC (2)
APC	N/A	<1	<1 - 29	<1	<1
Lm	N/A	N/A	N/A	N/A	N/A

2020 experiments (depth/surface sampling method)

n=3	Ham UL	Ham L (1)	Ham L (2)	Baloney L
APC	<1 - 53	<1 - 36	approx. 2 - 37	<1 - approx. 2
Lm	<1	<1	<1	<1

2019 experiments (swab sampling method)

n=3	Imprint	Ham M NIC (1)	Ham M IC (1)	Ham M NIC (2)	Ham M IC (2)
APC	<10	<1	<1	<1	1-2
Lm	<1	N/A	N/A	N/A	N/A

2020 experiments (swab sampling method)

n=3	Ham UL	Ham L (1)	Ham L (2)	Baloney L
APC	<1 - approx. 5	approx. 1 - 390	<1 - approx. 16	<1 - approx. 1
Lm	<1	<1	<1	<1

APC + Listeria counts (log CFU/cm^2) for the surface of spiked bulk product

2019 experiments (depth/surface sampling method)

n=3	Imprint	Ham M NIC (1)	Ham M IC (1)	Ham M NIC (2)	Ham M IC (2)
APC	N/A	N/A	N/A	N/A	N/A
Lm	N/A	approx. 5.3	approx. 5.3	6.0 ± 0.11	6.5 ± 0.10

2020 experiments (depth/surface sampling method)

n=3	Ham UL	Ham L (1)	Ham L (2)	Baloney L
APC	1.0 ± 0.08	3.3 ± 0.09	3.1 ± 0.12	3.8 ± 0.15
Lm	1.0 ± 0.00	3.4 ± 0.02	3.1 ± 0.11	3.8 ± 0.13

2019 experiments (swab sampling method)

n=3	Imprint	Ham M NIC (1)	Ham M IC (1)	Ham M NIC (2)	Ham M IC (2)
APC	N/A	N/A	N/A	N/A	N/A
Lm	6.3 ± 0.03	approx. 5.3	approx. 5.0	5.4 ± 0.21	5.9 ± 0.05

2020 experiments (swab sampling method)

n=3	Ham UL	Ham L (1)	Ham L (2)	Baloney L
APC	1.0 ± 0.33	3.3 ± 0.23	2.9 ± 0.10	3.8 ± 0.07
Lm	0.9 ± 0.15	3.3 ± 0.09	2.9 ± 0.09	3.6 ± 0.05

APC + Listeria counts for sliced product

Bacterial counts (APC and *Lm*) for slices of non-spiked bulk product (run prior to running the spiked bulk product through the slicer) and for slices of the spiked bulk product.

2020 experiments – bacterial counts (log CFU/g) for non-spiked product (before contaminating the slicer)

n=2	Ham UL	Ham L (1)*	Ham L (2)	Baloney L
APC	approx. 1.8 ± 0.3	N/A	4.0 ± 0.2	approx. 1.6 ± 0.0
Lm	<-1.4	N/A	<-1.4	<1

*Results not available due to an error during sampling

2020 experiments – bacterial counts (log CFU/g) for sliced spiked product

n=2	Ham UL	Ham L (1)	Ham L (2)	Baloney L
APC	3.0 ± 0.0	3.4 ± 0.1	4.1 ± 0.0	3.6 ± 0.1
Lm	<0.1	2.9 ± 0.1	2.6 ± 0.0	3.6 ± 0.1

Bacterial cells (log CFU/cm^2) in niches and on conveyor belt

Numbers in *red, italic* are approximate
Numbers preceded by '<' were not detected with that specific detection limit
Numbers preceded by '>' were too numerous to count on some of the plates

	Location	Crumbles on table under slicer blade		Crumbles by roller under conveyor belt		Conveyor belt (5 locations)	
	Lm/APC	*Lm*	APC	*Lm*	APC	*Lm*	APC
Ham UL	Clean[1)]	<-1.0	*<-1.0*	<-0.4	1.4	<-1.0	*0.8*
	Cont.[2)]	0.3	1.6	0.7	1.3	0.1	*1.0*
	During[3)]	0.3	1.7	0.7	2.1	-0.1	*0.6*
	After[4)]	0.1	1.1	0.9	2.2	-0.5	*<-0.8*
Ham L (1)	Clean	N/A[5)]	N/A	N/A	N/A	N/A	N/A
	Cont.	*0.3*	*0.6*	*0.9*	4.1	*0.8*	*1.8*
	During	0.6	1.0	1.3	3.8	-0.6	3.0
	After	-1.0	1.3	-0.4	4.0	-0.8	*3.4*
Ham L (2)	Clean	<-1.0	0.3	<-0.4	*>4.2*	<-1.0	*3.5*
	Cont.	*0.6*	*0.7*	*0.9*	*5.8*	*0.6*	*4.1*
	During	0.5	1.0	0.7	*5.2*	0.0	4.6
	After	0.3	2.8	0.7	4.0	0.0	4.3
Baloney L	Clean	<-1.0	*<-0.7*	<-0.4	*<-0.1*	<-1.0	*<-0.9*
	Cont.	1.6	1.9	*1.4*	*2.0*	*0.8*	*0.8*
	During	*0.6*	*0.6*	*0.9*	2.4	-0.7	*-0.1*
	After	1.0	1.3	-0.4	*1.0*	-0.2	*0.0*

1) After slicing approx. 200 slices of non-spiked product (before contaminating slicer with spiked product)
2) After slicing approx. 450 slices of spiked product
3) After slicing approx. 4,500 slices of non-spiked product (after contaminating slicer with spiked product)
4) After slicing approx. 11,000 slices of non-spiked product (after contaminating slicer with spiked product)
5) Data not available due to an error during sampling

Bacterial transfer – product to equipment

The slicer was swabbed in multiple locations (Appendix 1) before and after it was contaminated with the spiked bulk product. The difference between the before and after numbers reflects how many bacterial cells that are trapped in the various locations of the equipment.

Location	APC/ *Lm*	Ham ultra-low			Ham low (1)			Ham low (2)			Baloney low		
		Before	After	*Diff*	Before	After	*Diff*	Before	After	*Diff*	Before	After	*Diff*
Table under the slicer blade	*Lm*	-1.0	0.1	***1.0***	-1.0	-0.6	***0.4***	-1.0	0.1	***1.0***	-0.7	-0.1	***0.6***
	APC	-1.0	1.2	***2.2***	-0.1	0.8	***0.9***	-0.1	0.6	***0.7***	-0.7	-0.1	***0.6***
Edge of the slicer blade	*Lm*	-1.4	-0.3	***1.0***	-1.4	-1.1	***0.3***	-1.4	-0.1	***1.3***	-1.1	-0.8	***0.3***
	APC	-0.4	0.2	***0.5***	-1.4	1.0	***2.3***	-1.4	0.8	***2.2***	-1.4	-0.1	***1.3***
Enclosure behind blade	*Lm*	-0.9	0.1	***1.0***	-0.9	0.6	***1.5***	-0.9	0.1	***1.0***	-0.6	-0.9	***-0.3***
	APC	-0.9	-0.6	***0.3***	-0.9	1.0	***2.0***	-0.9	0.2	***1.1***	-0.9	0.1	***1.0***
Protective shield lower part	*Lm*	-0.9	0.2	***1.0***	-0.9	-0.6	***0.3***	-0.9	0.4	***1.3***	-0.6	-0.3	***0.3***
	APC	0.4	2.1	***1.7***	-0.9	0.9	***1.8***	-0.9	2.0	***2.8***	-0.6	0.9	***1.5***
Protective shield upper part	*Lm*	-1.3	-1.3	***0.0***	-1.3	-1.3	***0.0***	-1.3	-0.2	***1.0***	-1.0	-1.0	***0.0***
	APC	0.3	-0.7	***-1.0***	-1.0	-0.7	***0.3***	-1.3	1.4	***2.6***	-1.3	-1.0	***0.3***
Rails on feeding chute	*Lm*	-1,5	-0,4	***1,0***	-1,5	1,1	***2,6***	-1,5	-0,2	***1,3***	-1,2	-0,9	***0,3***
	APC	-0,4	1,2	***1,6***	-0,7	2,8	***3,5***	-1,5	1,3	***2,8***	-1,5	-1,2	***0,3***
Rim of enclosure	*Lm*	-1,1	-0,1	***1,0***	-1,1	-1,1	***0,0***	-1,1	0,2	***1,3***	-1,1	0,4	***1,5***
	APC	-0,5	1,8	***2,3***	-0,8	-0,3	***0,5***	-0,8	1,7	***2,5***	-1,1	0,3	***1,4***
Board at end of feeding chute	*Lm*	-0,6	0,4	***1,0***	-0,6	1,1	***1,7***	-0,6	0,7	***1,3***	-0,6	1,2	***1,8***
	APC	-0,6	1,5	***2,1***	-0,3	2,1	***2,5***	-0,6	2,0	***2,7***	-0,6	1,4	***2,0***

Numbers may be rounded