

Estimating undetectably low post-pasteurization recontamination levels of milk with pathogens using surrogate microbial variables

J. Hein M. van Lieverloo, J. Meeuwisse, A. Wagendorp, Martijn B. Fox and Marjon H.J. Wells-Bennik

NIZO food research, Kerhemseweg 2, 6718 ZB Ede, the Netherlands

Abstract

A model was constructed to estimate maximum recontamination probability via air during packaging of milk products. Based on ratios of Plate Count (PC) to estimated maximum (none found) *Listeria* levels in air samples and PC levels in the air in the filling machine, the estimated mean *Listeria* spp. contamination probability via air would be less than $3.4 \cdot 10^{-6}$ per carton. The air contamination model was validated using concentrations of Gram-negative bacteria in air samples and the frequency of spoilage with Gram-negative bacteria in daily test samples of 12 cartons taken from the filling machine. The validation shows that on some days more cartons are contaminated with Gram-negative bacteria than expected from the air contamination model, indicating the need for modeling other contamination routes.

Based on the ratios of PC to estimated maximum (none found) *Listeria* levels in surface swab samples and PC levels in the air in the filling machine, the most likely estimated maximum *Listeria* spp. contamination probability via air would be less than $1.7 \cdot 10^{-8}$ per carton. As no *Listeria* spp. was found in any of the samples, the actual probability of contamination is lower.

Keywords Packaging, air, Monte Carlo analysis, *Listeria monocytogenes*, total plate counts, Gram-negative bacteria..

Introduction

Recontamination of processed foods may form an important contribution to spoilage and the presence of pathogens in food. Few risk assessments and research projects however include the probability of recontamination (Reij & Den Aantrekker, 2004). Growth models show that *Listeria monocytogenes* readily grows in pasteurized milk (Te Giffel and Zwietering, 1996) and any contamination after pasteurization will lead to a high probability of exceeding the EU food safety objective of < 100 cfu/g at the time of consumption (Van Lieverloo et al., 2007). Contamination incidents such as the USA Whittier farm post-pasteurization outbreak end of 2007 show the need for rigorous control of any post-pasteurization recontamination (Anonymous, 2008). The dairy industry strives for extremely low probabilities of recontamination of milk, preferably zero. This poses the challenge of determining actual levels of recontamination. As it is not feasible to ever test for such low probabilities of finding *L. monocytogenes* in milk or milk products, a model for indirect assessment was constructed. From the possible points of recontamination identified during HACCP, two priorities were selected; (i) failing pasteurization control (as presented in another abstract of this conference) and (ii) recontamination via the air during packaging.

Materials and method

Air samples

The contamination routes were mapped for the oldest model of packaging machines available in the company (Figure 1). On 10 days from September to December 2008, air samples were collected on the roof (air-inlet into factory and filling machine), the environment of the filling machine, the mandrel compartment of the machine (blank folding and bottom sealing), the filling compartment and the top sealing compartment. Air samples were either analyzed for total plate counts (PC agar, Tritium Veldhoven, the Netherlands, 100-250 L air samples), Gram-negative bacteria (Tritium EC broth agar, 500-1000 L) and *Listeria* spp (Tritium PALCAM agar, 1000 L) using the Merck MAS-100[®] air sampler.

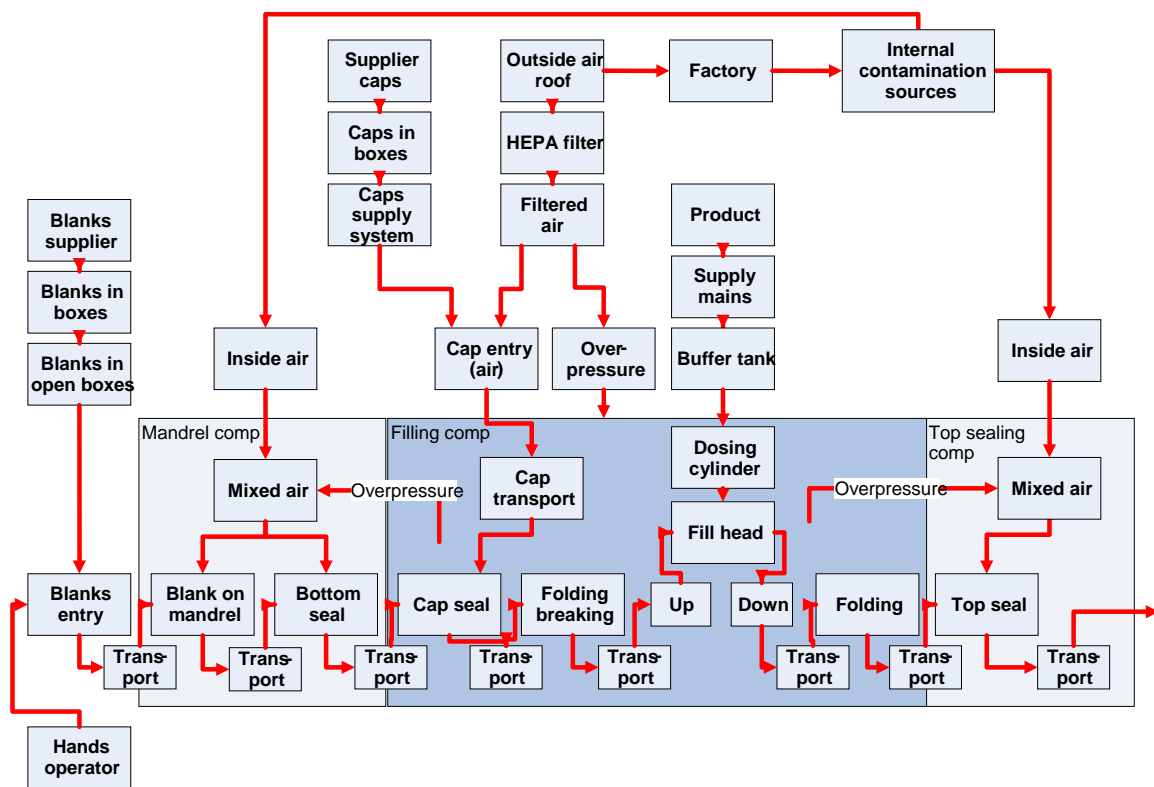


Figure 1: Schematic overview of the milk carton packaging machine and the possible contamination routes (only contamination via air was investigated).

Surface samples

In July 2009, surfaces (c. 100 cm²) in the factory were swabbed with a sterile cotton swab (wetted in 1.5 ml sterile water) and samples were kept on melting ice and in a 4 °C refrigerator before analysis (within 24 hours). After vortexing, sub-samples were transferred to agar plates to determine total plate count (PCA medium) and *Listeria* spp. (PALCAM agar, three plates with each 0.1 ml). The detection limit for *Listeria* spp. was 1 cfu per 20 cm².

Model

The model is described as

$$Lspp_{tot} = Poisson \sum_{i=1}^n \left(\frac{PC_{comp,i}}{PC_{ref,i}} * Lspp_{ref,i} * V_{comp,i} \right)$$

where

- Lspp = concentration of *Listeria* spp. (cfu/l)
- PC = concentration of plate count (cfu/l)
- V_{comp} = volume (l) of air that entered the carton per compartment
- tot = in carton
- comp = in compartment
- ref = on reference site

Three compartments and reference sites were distinguished

- Mandrel compartment with the filling machine environment as reference site
- Filling compartment with the roof as reference site
- Top seal compartment with the filling machine environment as reference site

Although the air in the mandrel and top seal compartment is a mixture of (HEPA filtered) air from the filling compartment and the filling machine environment, most of the bacteria in the air are likely to originate from the filling machine environment. The volume of air and deposition of air particles into the milk carton was calculated:

- 1225 ml of air: when the open carton is released from the bottom sealer (mandrel)
- equivalent of 7.9 ml of air (particles) deposited in the mandrel compartment
- equivalent of 15.9 ml of air (particles) deposited in the filling compartment
- equivalent of 6.6 ml of air (particles) deposited in the top seal compartment.

The deposition of particles in the open carton was calculated based on the deposition rate of 0,027 m/s determined by Den Aantrekker et al (2003) and the mean residence time in the parts of the filling machine. The model was validated using the frequency of milk carton contaminated with Gram-negative bacteria in daily test series.

The model was calculated in two forms, one using PC and *Listeria* spp. ratios from air samples in the filling machine environment and the other using PC and *Listeria* spp. ratios from swab samples in the filling machine environment.

The model based on air samples was validated using concentrations of Gram-negative bacteria in air samples and the frequency of Gram-negative contamination of 12 milk (product) cartons tested daily (and more often during air sampling days).

In the models, independence between all variables was assumed, probably overestimating contamination probabilities as high levels of plate counts probably are associated with the highest probability of finding *Listeria* spp.. As no *Listeria* spp. were found in any of the samples, the model is valid for a homogeneous air composition and therefore can not be used to estimate contamination during incidents.

Calculations

Data analysis was performed using @RISK 5.5 (Palisade) and Microsoft® Excel® 2002 (XP). The air model was calculated for the 10 individual sampling days in 2008 (100 simulations of 10,000 iterations each).

Results and discussion

Air samples

No *Listeria* spp. has been found in any of the 1000 L air samples on the roof (12), in the factory (46) and in the carton bottom sealing compartment (21). Assuming a homogeneous air composition and excluding any incident conditions, the concentration of *Listeria* spp. in the factory air is less than $2 \cdot 10^{-5}$ cfu/l ($\beta(1;47)/1000$; Vose, 2008).

Using the ratios of *Listeria* spp. to plate counts in the air and the plate counts in air in the filling machine, the mean probability *Listeria* spp. contamination via air in the filling machine was estimated to be less than $3.4 \cdot 10^{-6}$ per carton. The model predicts a daily variation of the contamination probability of less than 1 in 10.000 cartons in 81% to 99% of 100 simulations and less than 2 in 10.000 cartons in 98 %. Actual frequencies of contamination of 12 carton test series with Gram-negative bacteria on 4 of 10 sampling days were low but (statistically) significantly higher than predicted with the air contamination model, suggesting that other contamination routes remain to be evaluated.

Surface swab samples

Supplemental sampling for plate counts and *Listeria* spp. on surfaces in factories shows that on no site *Listeria* spp. was found, although high plate counts (PC; up to $2.1 \cdot 10^{-6}$) were found on sites where *Listeria* spp. was most likely to be found (floors, drains and wet conveyor belt housings). Based on the ratios of the highest PC to (theoretical maximum) *Listeria* levels in surface swab samples and PC levels in the filling machine, the most likely estimated mean *Listeria* spp. contamination probability would be less than $1.7 \cdot 10^{-8}$ per carton. As no *Listeria* spp. was found in any of the samples, the actual probability of contamination is lower. It also means that it is difficult to estimate the distribution of the recontamination probability.

Conclusion

This investigation shows that it is possible to estimate recontamination levels after pasteurization, although the absence of *Listeria* spp. in the filling machine environment leaves uncertainty about the actual contamination levels. The mean estimated frequencies of recontamination via air after pasteurization are around one in a million to one in 100 million cartons, but uncertainty about the probability distribution remains.

Literature

- Anonymous (2008) Environmental and milk products test positive for *Listeria*. Milk processing plant will remain closed until cleared by health officials. Press release January 17, 2008. The Official Website of the Office of Health and Human Services (EOHHS), Commonwealth of Massachusetts. http://www.mass.gov/?pageID=eohhs2pressrelease&L=1&L0=Home&sid=Eeohhs2&b=pressrelease&f=080117_whittier_farms&csid=Eeohhs2
- Den Aantrekker, E.D., Beumer, R.R., Van Gerwen, S.J., Zwietering, M.H., Van Schothorst, M. and Boom R.M. (2003) Estimating the probability of recontamination via the air using Monte Carlo simulations – Int. J. Food Microbiol. 87(1-2), 1-15
- Reij, M.W., Den Aantrekker, E.D. and ILSI Europe Risk Analysis in Microbiology Task Force (2004) Recontamination as a source of pathogens in processed foods. Int. J. Food Microbiol. 91, 1– 11
- Te Giffel, M.C. and Zwietering M.H. (1999) Validation of predictive models describing the growth of *Listeria*. Int. J. Food Microbiol. 46: 135-149
- Van Lieverloo, J.H.M., Fox, M., Schutyser, M., Te Giffel, M.C. and De Jong, P. (2007) Evolving from high through low uncertainty risk assessments for dairy products using kinetic, stochastic and fault tree modelling. Proc. 5th Int. Conf. Predictive Modeling in Foods, Athens, 16-19 September 2007.
- Vose, D. (2008) Risk analysis: a quantitative guide. 3rd edition. J. Wiley & Sons, Chichester, United Kingdom, 735 p.