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Detection of *Listeria* spp. and *L. monocytogenes* in pooled test portion samples of processed dairy products

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ABSTRACT

Listeria monocytogenes is a major foodborne pathogen. Testing multiple portions of the same final product is often required to verify the effectiveness of a food safety management system. Therefore, it will be advantageous to the laboratories to combine these test portions and process as one sample. However, combining samples for analysis, i.e., pooling, can be done only if there is no negative impact on the result. The objective of this study was to validate pooling of test portions for the detection of *L. monocytogenes* and *Listeria* spp. in dairy products as no scientific evidence currently exists to support this practice. Six representative matrices, namely, pudding, yogurt, brie cheese, 2% milk, ice cream and infant formula were spiked separately with stressed *L. monocytogenes* and *Listeria* spp. in 25 g and pooled test portions (375 g/250 g/125 g). Two methods, namely, ISO-11290-1:1996 Amd1:2004 and a validated alternative method Rapid'L.Mono were used for sample testing. Performance of a method in pooled test portions was considered to be satisfactory if the relative limit of detection ($RLOD_{50}$; LOD_{50} [pooled test portion]/LOD_{50} [25 g test portion]) and limit of detection (LOD_{50}) obtained was ≤ 2.5 and 1 CFU or MPN, respectively. Results obtained from *L. monocytogenes* and *Listeria* spp. trials were given equal weightage to decide on the impact of pooling. Acceptable $RLOD_{50}$ and LOD_{50} values were consistently obtained in *L. monocytogenes* and *Listeria* spp. inoculation experiments when test portions were pooled up to 125 g for all matrices tested with both methods. While there was a slight delay for the primary enrichment of the pooled test portions to reach the desired incubation temperature when compared to the 25 g test portions, it did not negatively impact the outcome when samples were pooled up to 125 g. Background organisms were in general present at low concentrations and did not seem to adversely impact the recovery of the target organism in 125 g samples. Thus, pooling of test portions to up to 125 g for the detection of *L. monocytogenes* and *Listeria* spp. by two culture methods in processed dairy products has been validated.

1. Introduction

Listeriosis remains an important foodborne disease with 2206 confirmed cases reported from 28 member states in Europe alone in 2015 (EFSA and ECDC, 2016). *Listeria monocytogenes* (LM) is the etiological agent of human listeriosis, a disease characterised by abortions in pregnant women and high mortality rates in susceptible individuals in an invasive infection (Vazquez-Boland et al., 2001). LM is mostly transmitted to humans through the consumption of contaminated high moisture ready-to-eat (RTE) and non-ready-to-eat (NRTE) foods manufactured in wet and cold processing facilities. Major source of infection includes contaminated RTE meat, fish and dairy products, though other foods of plant origin such as cantaloupes have been involved in some outbreaks. Food items from the processed dairy food category such as soft cheese are an important contributor of LM infections (EFSA and ECDC, 2016) and in the recent past a novel vector from this category, ice cream, has been incriminated in an outbreak in the United States (Rietberg et al., 2016). Though LM is mostly associated with high moisture products manufactured in wet and cold environment, it is also considered a significant pathogen in low moisture foods targeted for sensitive population such as infants and pregnant women.

Control of LM in finished products is achieved by a combination of stringent raw material microbiological specifications, application of validated kill steps, good manufacturing practices, hygienic design of facility and equipment, zoning, cleaning and sanitation programmes during manufacturing process and product handling steps in the

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downstream supply chain. Testing raw materials, environmental samples and finished products for LM and or its indicator *Listeria* spp. often verify the efficiency of these measures to control LM.

Depending on the product type and the regulatory framework in place, LM should be absent in finished products (absence in 25 g) or up to 100 colony forming units (CFU)/g is permissible in products, which do not support the growth of LM. For example, European Commission regulation no. 2073/2005 on microbiological criteria for foods requires absence of LM in 10 samples of 25 g ($n = 10$) for RTE foods intended for infants and special medical purposes and 5 samples of 25 g ($n = 5$) for RTE foods which supports the growth of LM. The legislation permits levels up to 100 CFU/g in RTE foods unable to support the growth of LM or if it can be demonstrated by the manufacturer that levels will not exceed 100 CFU/g throughout the shelf life of a product where growth can occur. The legislation also allows a manufacturer to set low intermediate limits during the process to ensure that the limit of 100 CFU/g is not exceeded at the end of the shelf life in products allowing LM growth (EC, 2005). Consequently, a typical sampling plan for LM qualitative (presence or absence) analysis requires testing of multiple 25 g test portions for products, which can result in extensive testing for the same product type. Therefore, for the qualitative testing of LM in multiple samples, strategies such as compositing and pooling can be used to reduce the associated analytical costs and to increase the laboratory efficiency.

When testing a composited sample, only a portion of the original laboratory sample is tested (Anonymous, 2017a) (Fig. 1). Compositing may have the greatest reduction on both analyst time and consumable cost, but it will also have a considerable impact on the reliability of the analytical result. Pooling is an alternative strategy for testing multiple samples and it comprises of two approaches: pooling of test portions ("dry pooling") and pooling of (pre-) enriched test portions ("wet pooling"). In the case of test portion pooling, the samples are pooled in the initial suspension. In the case of pooling (pre)-enriched test portions, aliquots of the primary enrichments are combined into a single secondary enrichment (Fig. 1). When pooling samples it is important to consider the impact if a positive result is obtained. For example, re-testing of individual samples may be required following a positive result to trace back the original contamination source and this may not be possible or provide the same result due to the low-level contamination of some samples. Therefore, ideally, only samples from the same batch/lot are pooled as a positive result for one or all samples will result in the same action.

Since legislation mandates the absence of LM in 25 g of finished product in a two-class sampling plan, detection methods are developed and validated to detect one target organism in a 25 g sample. Therefore, empirical evidence is required to assess the impact of compositing and pooling to ensure that these sample manipulations do not negatively affect the recovery of the target organism. This is of particular importance when testing for the presence of LM as it is well known that the recovery of LM can be negatively impacted by the presence of background organisms competing for growth such as *Listeria* spp. (Gnanou-Besse et al., 2010). This negative impact may have resulted from the production of inhibitors, nutritional competition, differences in growth rates as well as competition from non-*Listeria* background (Gnanou-Besse, Favret, Desreumaux, Decourseulles Brasseur, & Kalmokoff, 2016).

Several approaches are described in the literature to establish the impact of pooling matrices for pathogen detection. There are publications that assess the impact by comparing method performance characteristics such as sensitivity, specificity and efficiency (Vitas, Diez-Leturia, Tabar, & Gonzalez, 2014), limit of detection (LOD) and relative limit of detection (RLOD) (Tomas Fornes, McMahon, Moulin, & Klijn, 2017). Another approach is using a model measuring the growth during pre-enrichment to predict the impact of modifications, such as pooling, to the enrichment protocol. This was used for *Cronobacter* (Miled et al., 2011) and LM (Augustin et al., 2016) detection in pooled samples. In the international standard detailing the preparation of test samples, initial suspension and decimal dilutions for microbiology examination, ISO-6887-1:2017 (Anonymous, 2017a), a verification protocol to assess the impact of pooling on an analytical method is described.

However, only a limited number of scientific studies have been reported on the effect of test portion pooling for the detection of LM and *Listeria* spp. (Augustin et al., 2016; Becker et al., 2012; Curiale, 2000; Vitas et al., 2014). Curiale (2000) evaluated the performance of test portion pooling for the detection of LM in RTE meat and poultry products and found that there was no significant difference when samples

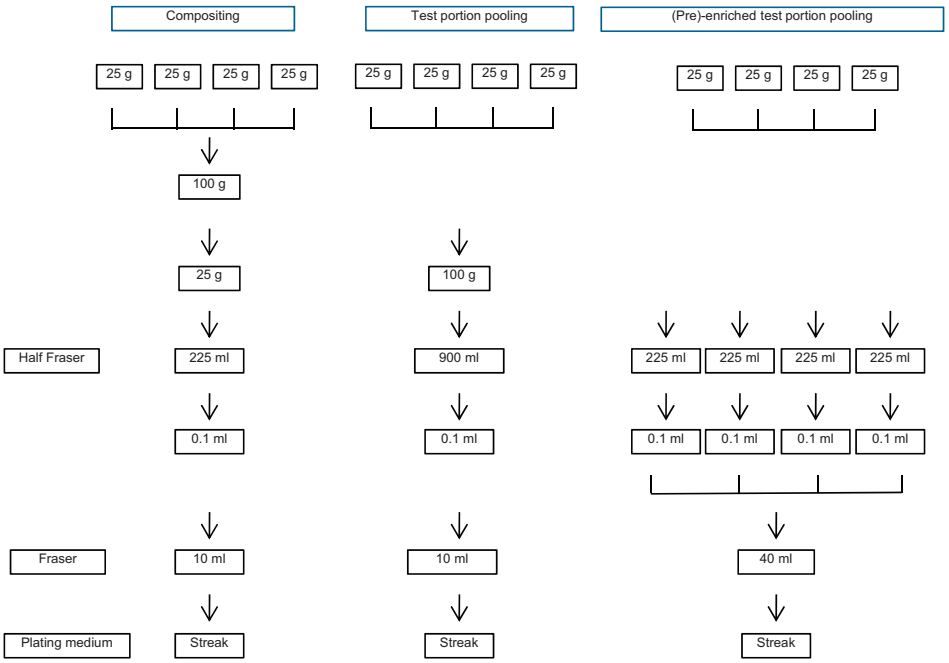

Fig. 1. Schema describing compositing, test portion (dry) pooling and (pre)-enriched test portion (wet) pooling. Test portion pooling was evaluated in this study where multiple 25 g samples were combined and processed as a single sample.