Comparison of Sampling Procedures for Recovery of *Listeria monocytogenes* from Stainless Steel Food Contact Surfaces

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ABSTRACT

A number of techniques exist for microbiological sampling of food processing environments in food industries. In the present study the efficacies of nine sampling procedures for the recovery of *Listeria monocytogenes* from food contact surfaces, including a new sampling device consisting of a miniroller, were evaluated and compared. A stainless steel table was inoculated with *L. monocytogenes* strain 935 (serovar 4b, human origin) and *L. monocytogenes* strain 437/07 (serovar 1/2b, food origin), at 10^5 CFU/100 cm^2*. *L. monocytogenes* strain 935 was best recovered with the minirollers (recovery of up to 6.27%), while poor recoveries (<0.30%) were obtained with the towel (one-ply composite tissue), alginate swab, metallic swab, and Petrifilm methods. In the case of *L. monocytogenes* strain 437/07 the replicate organism detection and counting (RODAC) ALOA contact plates yielded the best recoveries (4.15%), followed by the minirollers (up to 1.52%). Overall, recovery percentages with the minirollers were higher with stomacher homogenization than with Vibromatic agitation. The recovery percentages obtained for the *Listeria* strain of human origin were higher than those obtained with the food strain for all sampling procedures except Petrifilm and RODAC ALOA. With the miniroller device coated with wool fiber, the recovery of *L. monocytogenes* can be improved from 2 to 17 times over recoveries obtained with the sponge and cotton swab. This is the first report of a miniroller device for microbiological sampling in the available literature. The novel sampling procedure is convenient to apply on surfaces, is cost-effective, and results in better recovery of *L. monocytogenes* than do the conventional methods.

*Listeria* is a gram-positive, facultatively anaerobic, psychrotrophic, catalase-positive, and rod-shaped bacterium that can grow over a wide range of pH (4.6 to 9.6) (33) and temperature (−2 to 45°C) (4). *Listeria* can survive in the presence of high salt concentrations (above 20%, wt/vol) and low water activity down to 0.90 (23), being able to survive for 259 days at 4°C in cheese brine with NaCl concentration of 23.8% (24). This bacterium is introduced into the environment of food processing plants through animals, foods, and the materials of the facilities and can become widespread (19, 20).

*Listeria monocytogenes* is the most important pathogen associated with foodborne outbreaks because of the high mortality rate (20 to 30%) of the infectious disease it causes in at-risk groups (31, 38) and the long persistence and widespread distribution of the organism in the food processing environment (26, 39, 40). This foodborne pathogen is able to grow to populations of 10^5 to 10^6 CFU/g in many ready-to-eat chilled foods such as smoked salmon and sliced meat and meat products (18), and it can reach even higher populations in fluid milk and some types of soft cheeses (10, 29, 35). During the summer of 2008, *L. monocytogenes* caused an outbreak in Canada linked to ready-to-eat meat products, causing 22 deaths and 57 cases (17). In 2011, an important foodborne outbreak of listeriosis has been reported, linked to cantaloupes from one Colorado farm, with 146 people infected with *L. monocytogenes* and 29 deaths (8).

In food processing plants, there are places where spoilage and pathogenic microorganisms can grow and adhere to food contact surfaces and cavities inaccessible for cleaning and disinfection. High levels of organic matter facilitate the attachment of microorganisms to the surface areas (45). This adhesion is determined by the pH, time of contact, temperature, type of bacterium, type of contact surface, cellular density, and osmolarity (7). Biofilms protect bacterial cells from the environment (UV light), antibacterial agents (particularly disinfectants), heat, and bacteriophages (37), making bacteria more resistant to removal and creating sources of contamination of facilities, products, and food handlers (14). Numerous studies have shown that *L. monocytogenes* is able to attach and form biofilms on food contact surfaces such as polystyrene, glass, and stainless steel (5, 12), influencing the effectiveness of the environmental sampling for this pathogen.

A number of techniques exist for microbiological sampling of food processing environments in food industries, and the choice of the correct sampling procedure is decisive, though any method can have advantages and disadvantages. More commonly used approaches include the use of premoistened sponges, traditional swabs, and...
the accuracy and repeatability of experiments using cotton swabs and cellulose sponges for microbiological sampling of environmental surfaces have been debated since the introduction of these techniques in the 1970s. A rise in the efficiency of swabbing has been observed when dry and wet swabs are alternated in successive samplings (32). The degree of pressure exerted by swabbing is difficult to quantify, and it is influenced by the design of the swab (15). The sponges have been suggested as an alternative to swabs (27), though both sampling methods have relatively low recoveries of microorganisms, particularly when there are few bacteria (44). Other authors have suggested using contact plates for the sampling of firm and flat surfaces (28).

Another potential contributing factor that may influence the recovery of microorganisms is the material of the sampling device. For instance, due to the very porous structure of the sponge, the cells of Listeria can be entrapped in the matrix of cellulose during sampling (30) or during sample homogenization (27). In contrast, one-ply composite devices have a nonporous rough composition plus an inherent antistatic coating allowing for efficient recovery of L. monocytogenes from stainless steel surfaces (41). In some cases, the recovery of microorganisms increases when abrasive devices are used for sampling (13). Another factor to consider is the state of the surface, since the recovery from wet surfaces is usually higher than from dry surfaces (27), probably due to the loss of viability of the microorganisms by drying (11).

Published studies comparing the efficacies of different sampling methods for food contact surfaces have yielded conflicting results that are due to differences in experimental design. Vorst et al. (41) studied the recovery of L. monocytogenes from stainless steel surfaces using sponges, cotton swabs, alginate swabs, and one-ply composite tissues. Similarly, Kovačević et al. (21) compared the sponge, cotton swab, and a multilayer towel for the recovery of Listeria spp. in the environment of two meat processing facilities. Nyachuba and Donnelly (30) tested the effectiveness of 3M Petrifilm Environmental Listeria Plate and Microbial-Vac systems on the recovery of L. monocytogenes. Other sampling procedures that have been tested include the replicate organism detection and counting (RODAC) contact plates (15) and the so-called abrasive metallic swab (34).

The objective of the present study was to assess the efficacies of several environmental sampling techniques for quantitative recovery of L. monocytogenes from stainless steel food contact surfaces. A new technique based on miniroller devices is described for the first time for routine sampling in the food industry.

**MATERIALS AND METHODS**

**Preparation of the study area.** A stainless steel table (70 by 200 cm, type AISI 304 standard) was used as a model surface for testing the sampling techniques. The table, which had 10 years of use in food preparation and showed abrasions and scratches over the entire working surface, was used to duplicate conditions of a stainless steel surface in the food industry. Before each experiment the entire surface was cleaned and disinfected, and the same grids (10 by 10 cm; 100 cm²) were marked with a permanent marker and used for all replicate assays. The table was first washed with water and commercial soap (15 to 30% anionic surfactants, 5 to 15% nonionic surfactants, methylisothiazolinone, and phenoxethanol) and wiped three times with a clean damp cloth. Then, disinfection was performed by flooding with diluted bleach (0.67% in distilled water) for 2 min. Finally, the table was dried with sterile disposable paper, rinsed four times with sterile distilled water, and wiped with a sterile cloth.

**Bacterial strains.** Two strains of L. monocytogenes were used for contamination of the stainless steel grids: L. monocytogenes CECT 935 (serovar 4b) of human origin and L. monocytogenes 437/07 (serovar 1/2b) of food origin (isolated from fresh sausage without nitrates or nitrites). These strains were maintained in cryovials (Nirco S.L., Barcelona, Spain) frozen at −80°C in an ultralow temperature freezer (Sanyo Europe Ltd., Watford, Hertfordshire, UK).

**Surface contamination.** Each strain of L. monocytogenes was adjusted to a population of 10⁶ CFU/ml in brain heart infusion (Merck) measured by spectrophotometry (Spectronic 20, Bausch & Lomb), from a pure 12-h culture in the same medium. Then, an aliquot was added to a test fraction composed of 15 g of minced pork meat and 135 ml of 0.1% sterile peptone water and homogenized in a stomacher for 5 min with a strainer (filter) bag to remove particulate material. This suspension (pH 6.1) containing 10⁶ CFU/ml was stirred with a magnetic agitator (Agimatic-N, J. P. Selecta, Barcelona, Spain) at room temperature for 25 min, allowing for adaptation of L. monocytogenes to the new conditions. After this period, the center of each 100-cm² grid (10⁵ CFU/100 cm²) was inoculated with 0.1 ml of this suspension, which was uniformly spread with a sterile Drigalski glass spatula and allowed to air dry for 15 min. For verification that the minced pork meat was free of Listeria contamination, 20 samples were analyzed for L. monocytogenes by International Organization for Standardization methods ISO 11290-1 and 11290-2, obtaining results of <1.0 × 10⁴ CFU/g and absence of the organism in 25 g, respectively.

**Sampling methods studied.** The nine sampling procedures compared for recovery of L. monocytogenes from stainless steel are shown in Table 1. For all procedures, 100-cm² grid surfaces contaminated at 10⁵ CFU/100 cm² were assayed, except for RODAC plates, where 150-cm² grid surfaces contaminated at 10⁶ CFU/150 cm² were sampled. For each of the two Listeria strains, five different grids were inoculated from one single culture and assayed at the same time, totaling 10 experimental results for each sampling procedure.

**Premoistened sponge and premoistened towel (one-ply composite tissue).** Sponges (4 by 8 cm; HidraSponge [10 ml of buffered peptone water], 3M, Bricknell, UK) and towels (20 by 20 cm), folded to 50 cm² (AES Laboratory, Barcelona, Spain), were used according to the corresponding sampling instructions provided by the suppliers. Excess liquids were aseptically removed inside their packaging by pressing with sanitized latex gloves (washing with 70% isopropyl alcohol and rubbing to evaporation). The device was grasped, and a gentle but firm pressure was applied to cover the entire surface with 40 horizontal strokes in an overlapping “S” pattern. The device was turned over, and the same area was wiped again with 40 vertical S strokes. In addition, the edges were used to wipe the same area using 20 diagonal S strokes for each diagonal direction. Then, the devices were placed...
inside a stomacher bag, and 100 ml of sterile 0.1% peptone water (Difco) was added.

**Cotton swab, alginate swab, and metallic swab.** Three types of swabs (0.5-cm head diameter) were studied including cotton swab (Eurotubo Collection Swab, Deltalab, Barcelona, Spain), alginate swab (Copan, Murrieta, CA), and custom-fabricated metallic swab (34). In the case of the alginate swab, a solution of 1% trisodium citrate dihydrate was used in order to dissolve the alginate. The abrasive metallic swabs were made in our laboratory with 25-cm-long strands of stainless steel wool taken from spiral scourers wound around the cotton bud of a standard swab, which were then autoclaved at 121°C for 15 min before use.

Four swabs were used per sampling (one swab for the vertical direction, another for the horizontal direction, and the others for the two diagonal directions), which were premoistened by dipping in 0.1% sterile peptone water and draining excess fluid. Thereafter, the surface was similarly sampled (40 swabbing strokes in each direction) in a rolling motion with rubbing to maximize swab contact. The swabs were placed and cut inside a stomacher bag, and 100 ml of sterile 0.1% peptone water was added.

**Cotton disk and cotton gauze.** Sterile cotton disks of 5.5-cm diameter (AS Schlecker, Ehingen, Germany) and cotton gauze pads (28.6 by 14 cm), folded to 50 cm² (Lusan, Unitec-Hartmann Laboratories, Barcelona, Spain), were aseptically removed from their packaging and disposed over the sampling area in a wiping pattern similar to that described above for the premoistened sponge and towel (in horizontal, vertical, and diagonal directions, alternating the exposed sides until the total surface of each device was used), moistening the stainless steel surface three times with 0.5 ml of sterile 0.1% peptone water. Then, the devices were placed inside a stomacher bag, and 98.5 ml of sterile 0.1% peptone water was added.

**Minrollers.** Five models of minrollers (Rulo Pluma SA, Nespoli Group, Barberà del Vallès, Barcelona, Spain), originally designed for painting, were used for environmental sampling. According to the literature review, this is the first report of such a device being used for microbiological environmental sampling. The minroller consists of a polypropylene tube with a length of 60 mm and a diameter of 16 mm (sampling surface of 30 cm²) and covered by materials of different composition for the five models tested (Table 1). Before use, the minrollers were washed with soap, rinsed with distilled water, autoclaved at 121°C for 15 min, and dried in an oven at 45°C for 12 h. The device handle was disinfected with 70% isopropanol and flamed before being attached to the minroller. The table grids were moistened three times with 0.5 ml of sterile 0.1% peptone water before the minrollers were used, rolling 10 times in each of the horizontal, vertical, and two diagonal directions. Then, the minrollers, including the tubes, were placed inside a stomacher bag, and 98.5 ml of sterile 0.1% peptone water was added.

**Steel wool pad.** The steel wool pads were prepared in our laboratory with 1 g of spiral strands of stainless steel wool fiber (obtained from commercial pan scourers) pressed into a circular mold of 18-mm diameter to form molded scouring pads, which were autoclaved before use at 121°C for 15 min. The sampling surface was moistened three times with 0.5 ml of sterile 0.1% peptone water, and the pads were rubbed in the horizontal direction (40 strokes), the vertical direction (40 strokes), and the two diagonal directions (40 strokes in each one) with sterile dissecting forceps, and the remaining liquid on the table grids was absorbed with a cotton disk. The pads of steel wool and the corresponding cotton disk were placed inside a stomacher bag, and 98.5 ml of sterile 0.1% peptone water was added.

**Petrifilm system.** The Petrifilm system (Environmental Listeria Plate, Petrifilm, 3M) was used following the manufacturer’s instructions. The commercial Petrifilm kit includes plates, a diffuser for spreading, and rayon-tipped swabs premoistened with listerine broth (1 ml). For each sampling area, three swabs were rubbed firmly across the table surface, with 40 strokes in each of the horizontal, vertical, and two diagonal directions. Then, the swabs were placed into tubes with 1 ml of listerine broth and combined in a stomacher bag with 57 ml of sterile buffer peptone water.

**RODAC plates.** Contact plates (5.5-cm diameter, 24 cm²) were prepared with ALOA agar (Oxoid, Ltd., Madrid, Spain) in a laminar flow chamber. The sampling was done by uncovering the plate and applying the agar surface to the grid under a pressure of 500 g (20 g/cm²) for 10 s (3). Preliminary assays indicated that when the contamination was above 10³ CFU/150 cm² the plates were completely covered by colonies, so that the L. monocytogenes inoculation level needed to be no greater than 10³ CFU/150 cm².

**Homogenization, plating, and counting.** Stomaching (IUL Masticator, Barcelona, Spain) for 5 min at 1,500 rpm was used for all procedures except the RODAC plates. Arm shaking (Vibromatic, J. P. Selecta, Barcelona, Spain) for 10 min at 764 vibrations per min was also used with the five models of minrollers for comparison with the stomacher assays. Duplicate plates of ALOA agar were surface spread with 0.25 ml of inoculum. The incubation was performed at 37°C, and counts were made at 24 to 48 h. In the case of the Petrifilm system, 3 ml was seeded directly into the plates and evenly homogenized with the large flat spreader for Listeria (Petrifilm, 3M). Results were expressed as mean recovery percentages ± standard deviations of the counts obtained in five replications, calculated as the number of L. monocytogenes cells.
TABLE 2. Performances of different sampling procedures for recovery of L. monocytogenes on stainless steel food contact surfacesa

<table>
<thead>
<tr>
<th>Device</th>
<th>L. monocytogenes strain 935 (human origin)</th>
<th>L. monocytogenes strain 437/07 (food origin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sponge</td>
<td>2.81 ± 0.63 BCD</td>
<td>0.97 ± 0.36 BCD</td>
</tr>
<tr>
<td>Premoistened towel</td>
<td>0.28 ± 0.18 G</td>
<td>0.06 ± 0.03 F</td>
</tr>
<tr>
<td>Cotton swab</td>
<td>1.01 ± 0.47 EFG</td>
<td>0.09 ± 0.07 F</td>
</tr>
<tr>
<td>Alginite swab</td>
<td>0.22 ± 0.15 G</td>
<td>0.14 ± 0.11 F</td>
</tr>
<tr>
<td>Metallic swab</td>
<td>0.24 ± 0.29 G</td>
<td>0.03 ± 0.03 F</td>
</tr>
<tr>
<td>Cotton disk</td>
<td>2.53 ± 0.96 CDEFG</td>
<td>1.22 ± 0.67 BCD</td>
</tr>
<tr>
<td>Cotton gauze</td>
<td>0.40 ± 0.15 G</td>
<td>0.35 ± 0.20 DEF</td>
</tr>
<tr>
<td>MR1S</td>
<td>3.53 ± 1.17 BCD</td>
<td>1.30 ± 0.72 BCD</td>
</tr>
<tr>
<td>MR2S</td>
<td>6.27 ± 1.62 A</td>
<td>1.52 ± 0.37 B</td>
</tr>
<tr>
<td>MR3S</td>
<td>3.31 ± 0.10 BCD</td>
<td>1.41 ± 0.55 BC</td>
</tr>
<tr>
<td>MR4S</td>
<td>2.32 ± 0.48 CDEFG</td>
<td>0.43 ± 0.17 CDE</td>
</tr>
<tr>
<td>MR5S</td>
<td>3.69 ± 0.64 BCD</td>
<td>0.59 ± 0.24 BCDE</td>
</tr>
<tr>
<td>MR1V</td>
<td>1.79 ± 0.36 DEFG</td>
<td>0.31 ± 0.12 DEF</td>
</tr>
<tr>
<td>MR2V</td>
<td>5.05 ± 2.19 AB</td>
<td>1.49 ± 0.65 B</td>
</tr>
<tr>
<td>MR3V</td>
<td>4.23 ± 1.53 ABC</td>
<td>0.21 ± 0.18 EF</td>
</tr>
<tr>
<td>MR4V</td>
<td>1.90 ± 0.58 CDEFG</td>
<td>0.61 ± 0.33 BCDE</td>
</tr>
<tr>
<td>MR5V</td>
<td>1.33 ± 0.66 DEFG</td>
<td>0.61 ± 0.46 BCDE</td>
</tr>
<tr>
<td>Steel wool pad</td>
<td>0.75 ± 0.44 FG</td>
<td>0.31 ± 0.15 DEF</td>
</tr>
<tr>
<td>Petrifilm</td>
<td>0.25 ± 0.29 G</td>
<td>1.20 ± 0.36 BCDE</td>
</tr>
<tr>
<td>RODAC ALOA</td>
<td>2.50 ± 1.29 CDEFG</td>
<td>4.15 ± 1.03 A</td>
</tr>
</tbody>
</table>

a Values are recovery percentages ± standard deviations. Values followed by different letters in a column are significantly different (P < 0.05). See Table 1 for material composition and abbreviations used for sampling devices.

RESULTS AND DISCUSSION

Table 2 shows the recovery percentages obtained with the two Listeria strains by the different sampling procedures. L. monocytogenes strain 935 of human origin (serovar 4b) was best recovered (more than 3%) by using minirollers MR1S, MR2S, MR3S, MR5S, MR2V, and MR3V, while poor recoveries (less than 0.30%) were obtained with the towel, alginate swab, metallic swab, and Petrifilm. The miniroller MR2 (coated with 100% wool fiber–velour) showed the highest recoveries among all minirollers. Considering all the miniroller types, the stomacher yielded higher recoveries than the Vibromatic for MR1, MR2, and MR3, but the difference was only significant for MR3, whose recovery percentage with the stomacher (1.41%) was significantly higher than with the Vibromatic (0.21%). The procedures that showed lower recovery percentages for strain 437/07 were the towel and the three types of swabs, probably indicating that a percentage of the recovered Listeria cells were not released from the devices into the diluent during homogenization. In contrast, the best yields obtained with RODAC ALOA plates in our study (4.15 ± 1.03%) indicate its suitability for the recovery of low Listeria populations (near 10³ CFU/150 cm²) from environmental surfaces.

Overall, the recovery percentage of the L. monocytogenes strains was not directly related to the size of the sampling device. For instance, the novel miniroller devices with a sampling surface area of 30 cm², together with the sponge (32 cm²) and RODAC contact plates (24 cm²), were more effective than the cotton disk (24 cm²), cotton gauze pad (50 cm²), towel (50 cm²), and swab. Likewise, the abrasive metallic swab and steel wool did not increase recoveries compared with the traditional cotton swab and cotton disk, respectively. According to our results the swabbing was not very effective, even though the use of four swabs was expected to increase recoveries in comparison to a single swab, which would become saturated. Among the minirollers, the most suitable material composition for the physical removal of the microorganisms was the wool fiber, followed by the polyamide fiber, white microfiber, and foam.

The recovery percentages obtained for Listeria strain 935 of human origin were higher than those obtained for the...
meat-derived *Listeria* strain 437/07 with all sampling procedures except Petrifilm and RODAC ALOA. These differences between isolates were statistically significant (*P* < 0.05) for the sponge, towel, cotton swab, all miniroller types, and Petrifilm. These results may indicate that the strain of food origin tends to attach more effectively to the food contact surface, which inversely influences bacterial recovery. It is well known that different serotypes of *L. monocytogenes* may behave differently depending on the strain source. For instance, several studies have shown higher prevalence of *L. monocytogenes* serotypes 1/2c and 1/2b in food processing environments, while serotype 4b strains occur more frequently in listeriosis outbreaks. López et al. (25) isolated *L. monocytogenes* from a pig abattoir environment and reported that 53 and 21% of strains were of serotype 1/2c and 1/2b, respectively. Similarly, Boerlin et al. (6) reported that serotypes 1/2a and 1/2b were more frequently isolated (63.8%) than serotype 4b (5.6%) from food or fish products. In another study, *L. monocytogenes* serotypes 1/2a, 1/2b, and 1/2c accounted for 90% of all strains isolated from foods (16). In summary, some strains of *L. monocytogenes* may be more adapted to food processing environments than others due to their ability to adhere to food contact surfaces and their higher resistance to disinfectants used in the food industry. Consequently, these *Listeria* strains might be able to outcompete other strains in the food processing environment (42).

Conventional methods for *L. monocytogenes* detection on food contact surfaces in the food industry include the premoistened sponge and towel as well as the swabs, Petrifilm, and contact plates. It is worth noting that the widely used methods of premoistened sponge and Petrifilm showed intermediate recoveries in our study. However, Nyachuba and Donnelly (30) found that the sponge and Petrifilm systems showed higher recoveries of *L. monocytogenes* from environmental surfaces than those obtained by the Microbial-Vac system, concluding that the sponge and Petrifilm systems are suitable for the recovery of low numbers of damaged *L. monocytogenes* cells from environmental surfaces. Our results indicated that RODAC ALOA plates recovered 4.15% of *L. monocytogenes*, which is somewhat lower than the 7% for *Campylobacter jejuni*, 18% for *Bacillus cereus*, and 23% for *Salmonella Enteritidis* reported by Kusumaningrum et al. (22) with RODAC plates on stainless steel surfaces.

As shown by several authors, the swab procedure has variable recovery rates for artificially inoculated microorganisms on hard metal surfaces and other materials (1, 2, 36). Vorst et al. (41) compared the sponge, cotton swab, alginate swab, and one-ply composite tissue for the enumeration of normal and damaged cells of *L. monocytogenes* from stainless steel surfaces and reported that one-ply composite tissue allowed for higher recoveries. Yan et al. (44) also obtained better recoveries of *L. monocytogenes* from food contact surfaces with premoistened one-ply composite tissues than with the sponge. Another study found that adhesive tape recovered more bacterial cells from solid surfaces than the cotton swab, suggesting that it could be used for routine monitoring of environmental quality (43).

The present study showed that a novel miniroller device specially coated with wool fiber–velour yielded higher recoveries of *L. monocytogenes* after stomaching than did the aforementioned conventional methods and may provide another, lower-cost, and effective alternative to the above. Regulation (EC) 2073/2005 (9) requires that food business operators manufacturing ready-to-eat foods that may pose an *L. monocytogenes* risk for public health shall sample the processing areas and equipment as part of their sampling scheme. Food business operators may use new and alternative sampling and testing procedures, if they can demonstrate that these procedures provide guarantees at least equivalent to those of the conventional methods. According to our results the miniroller coated with wool fiber provides results superior to those obtained by conventional procedures, so we feel that we can propose this new procedure as an effective alternative for recovery of *L. monocytogenes* from food contact surfaces.

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