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PEER-REVIEWED ARTICLE

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Experimental Evaluation of Performance of Sampling Techniques for Microbiological Quantification on Carcass Surfaces

ABSTRACT

Microbiological quantification methods are applied in European abattoirs for evaluating carcass hygiene. Sampling may influence the results and the aim of this study was to evaluate sampling methods for microbiological quantification of slaughter hygiene. Based on these results, a sampling technique in a subsequent Europe-wide baseline study would be selected. To ensure comparable results, a laboratory carcass model was developed from lamb flanks contaminated with a pre-defined inoculum. The SimPlate method for *Enterobacteriaceae* and *Escherichia coli* was used. The study consisted of three sections: pilot study (n = 36), two inocula, three sampling times (2 h, 12 h, and 24 h post-inoculation); main and confirmatory study: one inoculum level (n = 96); main study: four swabbing techniques (100 cm^2), two sampling times (2 h and 24 h), compared with a destructive sampling method (5 cm^2). The recovery of *Enterobacteriaceae* and *E. coli* was in the order: destructive > gauze cloth swab ≥ spongestick ≥ sponge swab > wet-dry double-swab. Although the destructive excision method resulted in the highest recovery of *Enterobacteriaceae* and *E. coli* in this study, other factors must also be considered for selecting a method for routine carcass sampling. Thus, gauze cloth swabbing was judged to be the most appropriate technique.

INTRODUCTION

The European meat industry is required to have testing regimes to monitor hygiene and to ensure the safety of meat. There are two requirements for the European meat industry: Hazard Analysis Critical Control Point (HACCP) programs and microbial performance standards *(15)* expressed as microbiological criteria *(15, 17)*. The European Food Safety Authority's (EFSA) Scientific Opinion on meat inspection describes a new meat inspection framework *(18)* in which EFSA recommended that an abattoir's ability to maintain good general hygiene practices should be measured by data trends derived from process hygiene assessments and from HACCP programs *(18)*. Hygiene categorization of the abattoirs based on these trend analyses could be used as documentation and benchmarking towards new and existing markets. For example, the ongoing Transatlantic Trade and Investment Partnership negotiations between the EU and the U.S. has reduction of technical barriers to trade as its main target; a benchmarking system for hygiene could be one approach for abattoirs to remain competitive in the future.

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Two classes of methods are used for sampling carcasses, destructive and non-destructive. The reference method of sampling for microbiological criteria regarding total bacterial counts and *Enterobacteriaceae* before chilling is a destructive method *(26)*. However, regulation EC No 2073 (2005) states that food business operators may use other methods, if they can demonstrate that these procedures provide at least equivalent performance *(17)*. In the U.S., swabbing is the common sampling technique. In Norway also, the national guidelines for good hygienic slaughter practices recommend swabbing of carcasses for sampling for *Escherichia coli* after chilling *(1)*. Regulation 2073/2005 refers to the standard International Organization for Standardization (ISO) 17604 "Microbiology of the food chain — Carcass sampling for microbiological analysis," which lists the different destructive and non-destructive sampling techniques, sampling sites, and rules for sample storage and transport *(17, 26)*. The diagnostic value (i.e., sensitivity, specificity, precision, and predictive value) of the still widely-used sampling techniques are not documented in the available literature *(25)*, and an official quantitative conversion factor between destructive and non-destructive methods has not yet been established *(4)*. A wide array of sampling techniques is currently used in the European meat industry, and it is therefore difficult both to evaluate their value in use and to make comparisons of results from different abattoirs and countries. The destructive method has been compared with swabbing techniques (non-destructive methods) in other studies; before chilling of pig carcasses *(29)* and after chilling of beef *(9)* and pig carcasses *(11)*. These studies resulted in approval for use of a swabbing technique in Denmark by the Danish Veterinary and Food Administration *(37)* and subsequent approval from the Norwegian Food Safety Authority for use in Norway *(33)*.

The destructive method of excision harvests almost all bacteria on the surface but reduces the commercial value of the carcass. Excision further requires more equipment (sterile knife, forceps, etc.) and skills/experience and is more time consuming, and it therefore might be less practical for routine carcass sampling *(8)*. Swabbing is a preferred technique in many abattoirs because it is non-destructive, enables sampling of larger areas of the carcass than excision, and might be more reliable when the level of total contamination is low and heterogeneously distributed on the carcass *(4)*. However, swabbing is subject to several possible sources of error due to operator variability. Bacterial recovery with swabbing increases with swab material abrasiveness *(4, 8)*, and coordination of the methodology and experimental design (swabbing materials, stage and time of sampling, size and location of the sampled area, microbiological analyses, etc.) is recommended in order to evaluate the performance of different carcass sampling techniques *(4)*. Published studies have mainly focused on individual steps and have been based on randomly selected, naturally contaminated carcasses. Carcasses are big, expensive, and difficult to handle; furthermore, natural contamination is distributed heterogeneously. Natural contamination of carcasses with *E. coli* is normally very low, and it is therefore difficult to study the recovery from sampling procedures, especially on chilled carcasses. The work described here is part of a research project (Hygenea), in which the aim is to ensure yield and payback from hygiene investments and achievements by developing tools for risk-based assessment of hygiene in abattoirs. The aim of this study was to evaluate five sampling techniques for quantitative microbiological characterization of slaughter hygiene of carcasses, before and after chilling, and to identify an acceptable and applicable non-destructive technique to be used in a subsequent baseline study.

MATERIALS AND METHODS

General experimental design

The study was implemented in three stages: a pilot experiment, a main experiment, and a confirmatory experiment. For all stages, samples were randomized.

In the pilot experiment, two levels of inoculum, two sampling techniques (destructive method (A) and gauze cloth swab (B)), and three sampling times were used (12 combinations). Each combination was run in triplicate, resulting in a total of 36 samples.

The (second) main experiment focused on the sampling techniques and used one inoculum level, five techniques and two sampling times (10 combinations), with six replicates. The main dataset thus consisted of 60 samples.

The (third) confirmatory experiment concentrated on the three sampling techniques that had the highest recovery of bacteria in the main experiment, one inoculum level and two sampling times (six combinations). Each combination was run in six replicates, resulting in a total of 36 samples.

For each experimental combination in all three sub-studies, non-inoculated controls were also analyzed. All three experiments (pilot, main, and confirmatory study) were conducted in one laboratory (at Animalia), and the preparation of inocula and the microbial analyses were all conducted at the Norwegian Veterinary Institute.

Assembly of the carcass model

A polypropylene drainage pipe with an outside diameter of 160 mm was cut into 4 cm lengths that were used as meat support frames for the carcass model. Flanks from sheep (*M. obliquus externus abdominis*) were removed less than 1 h post mortem at a local abattoir, put in plastic-covered crates and transported to the laboratory within an hour. To make the carcass model, a flank was placed over one end of the pipe frame, with the outer surface of the meat facing outward and fastened tightly in place with the aid of two or three cable ties *(Fig. 1)*. The flanks had an approximate thickness of 3–5 mm. The meat tissue carcass model was then inoculated and sampled at the designated time as described under "Preparation of inocula." For each technique, a non-inoculated control sample was used.

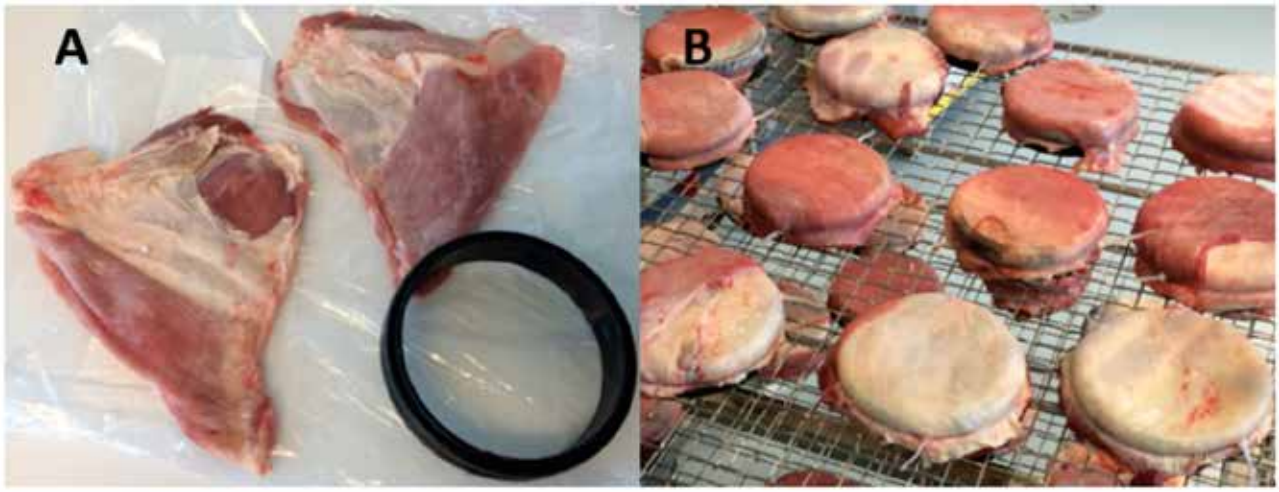

Figure 1. The carcass model. A: Flanks from sheep (M. obliquus externus abdominis) *were placed over the pipe frame. B: The flanks were placed with the outer surface of the meat facing outwards, and attached tightly in place over the pipe frame with the aid of two or three cable ties.*

Preparation of inocula

The inoculum used at different dilutions was comprised of three strains of *Enterobacteriaceae* associated with sheep carcasses *(34)*, namely *Citrobacter freundii* (CCUG 418), *Enterobacter cloacae* (CCUG 6323), and *Escherichia coli* (CCUG 17620). The strains were kept in glycerol stocks at below -75°C. Prior to preparation of the inoculum, the strains were plated from the stocks on blood agar (BA) plates and incubated overnight at 37°C ± 1°C. One well-isolated colony from each strain was inoculated separately into 9 mL of buffered peptone water and incubated at 37° ± 1°C for 24 h. For estimation of the number of bacteria, the cultures were serially diluted in peptone salt diluent (0.1% peptone, 0.9% NaCl) and 100 µl from the appropriate dilutions were plated onto BA in triplicate. In order to achieve the required concentration, an equal volume of the appropriate concentrations for each strain were mixed and kept on ice until use. For the pilot study, two inocula were prepared; (high) 2×10^6 colony-forming units (CFU)/mL, and (low) 2×10^3 CFU/mL. For the main and confirmatory experiments, inocula of estimates of moderate concentrations were used, 2.6×10^4 CFU/mL and 2.8×10^4 CFU/mL, respectively.

In all three experimental rounds, 500 µL was inoculated onto the surface of each meat tissue model and spread out evenly on an area of 100 cm^2, using a sterile L-shaped spreader. Inoculated samples were left on the bench to dry for a few minutes, before they were either a) sampled 2 h after inoculation, without chilling or b) stored for 12 h at 4 ± 0.5°C (only the pilot) or c) stored for 24 h at 4 ± 0.5°C.

Sampling of the carcass model

The sampling techniques investigated in this study were chosen on the basis of the techniques used by fifteen different abattoirs from EU and EEA countries participating in the Hygenea project. A specially made, circular cutting support board (approximately 130 mm in diameter and 4 cm thick) that fitted within the model pipe frame was used to ensure that the meat model did not dip into the hollow pipe during sampling.

After sampling, the excised meat samples and the swabs were placed in separate stomacher bags or tubes and kept refrigerated (4 ± 0.5°C) until being transported to the laboratory at the Norwegian Veterinary Institute for microbiological analysis the following day.

Destructive method; excision (A). A circular incision (25 mm in diameter) was made in the surface of the meat tissue model with a sterile coring tool with a circular blade (BeefSteaker, Bürkle, Germany) *(Fig. 2A)*. The disc of tissue (approximately 5 cm^2 and 2 mm thick) was removed from the tool blade with a sterile scalpel and forceps and placed in a labelled sterile stomacher bag.

Non-destructive methods. For all four of the non-destructive methods used, the test area on the carcass model was delineated by using a single-use, square template frame made of paper (100 cm^2) that was laid over the model, and the area within the template frame was sampled.

Gauze cloth swab (B). One sterile medical gauze cloth swab (10 × 10 cm) (Mesosoft, Mölnlycke Health Care AB, Sweden) was added to 10 mL sterile peptone salt diluent. The test area defined by the template was swabbed, using 10 horizontal and 10 vertical movements (approx. 20 s) *(Fig. 2B)*.

Sponge swab (C). The test area defined by the template was swabbed with a pre-moistened Polywipe (5 × 10 cm) (Medical Wire & Equipment, Wiltshire, UK), using 10 horizontal and 10 vertical movements (approx. 20 s) *(Fig. 2C)*.

Sponge-Stick (D). The test area defined by the template was swabbed with a pre-moistened Sponge-Stick (3.75 × 7.5

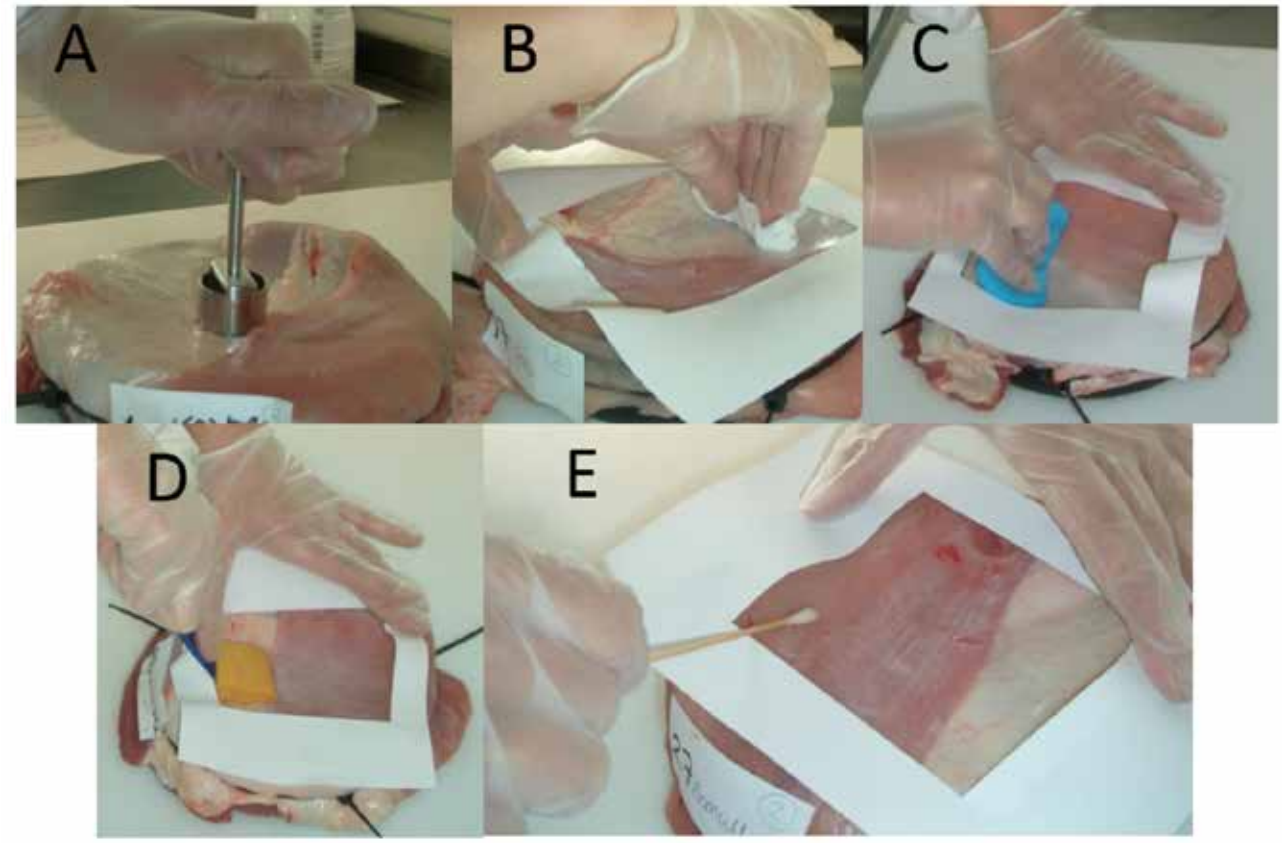

Figure 2. The different sampling techniques investigated. A: destructive method, B: gauze cloth swab, C: sponge swab, D: sponge-stick, E: wet-dry double swab.

cm) (3M Health Care, St. Paul, USA), with 10 horizontal and 10 vertical movements (approx. 20 s) *(Fig. 2D)*.

Wet-dry double-swab technique (E). A cotton swab with wood applicator, regular tip (approx. 1 cm × 0.5 cm) (Copan, Brescia, Italy), was dipped into 10 mL sterile peptone salt diluent. The wet cotton swab was rubbed over the whole test area twice. A dry cotton swab was rubbed at a 90° angle to the direction of the first rub, to absorb as much of the solution as possible *(Fig. 2E)*. Both swabs were put into 10 mL sterile peptone salt diluent in 50 mL tubes and further processed, described as follows.

Microbial analyses

Upon arrival at the laboratory, the samples were processed by adding 20 mL peptone salt diluent to each sample prior to homogenization for 30 s with a stomacher (Laboratory blender, Stomacher 400, Seward, UK). The samples from the wet-dry double swab technique were vortexed for 30 s. *Enterobacteriaceae* and *E. coli* were enumerated using SimPlate® *Enterobacteriaceae* (BioControl Systems Inc., Bellevue, WA, USA) and SimPlate® Coliforms/*E. coli* (BioControl Systems Inc., Bellevue, WA, USA). One mL from the appropriate dilution was placed in the center of the SimPlate plating device, 9 mL of a mixed nutrient agar with blue color was added at the same spot, and the device was rotated according to the manufacturer's instructions. The plates were incubated at 37 ± 1°C for 24–28 hours and read according to the manufacturer's instructions. For the *E. coli* analysis, wells that changed color from that of the background and that fluoresced when exposed to 366 nm UV light were counted as positive, and results were converted into most probable number (MPN) of *E. coli* per swab/sample according to a counting range conversion table. Correspondingly, for the *Enterobacteriaceae* analyses, wells with a color change from the background were counted as positive, according to the manufacturer's instructions. If all 84 wells demonstrated a positive reaction, the result was reported as >738 MPN per plate.

Statistical analyses

Randomization of the samples was accomplished in the Design of Experiment (DOE) module of JMP (JMP for Windows, ver 12, SAS Institute, Cary NC). Databases were established in Excel®, where data checking and primary analyses were done using the filter and pivot functions. The data were transformed from CFU per sample to $\log_{10}$ CFU

TABLE 1. Reduction in *Enterobacteriaceae* and *E. coli* in the main experiment by using median regression model. There were five sampling techniques (A–E), two sampling times (2 and 24 h) and one inoculum level (10^4). The regression coefficients described the change in $\log_{10}$ CFU per cm^2 for either *Enterobacteriaceae* or *E. coli* as an explanatory variable changed by one unit, the other variables being held constant. Baseline levels for the categorical variables were sampling technique A and 2 h period between inoculation and sampling.

Variable	*Enterobacteriaceae* ($\log_{10}/cm^2$)		*E. coli* ($\log_{10}/cm^2$)	
	Coefficient (95% CI)	*P*-value	Coefficient (95% CI)	*P*-value
	Time = 2 hours			
Destructive method (A)	0.00 (-)[1]	-	0.00 (-)[2]	-
Gauze cloth swab (B)	-0.34 (-0.68/0.03)	0.05	-0.49 (-0.71/-0.26)	< 0.001
Sponge swab (C)	-0.74 (-1.03/-0.45)	< 0.001	-0.50 (-0.73/-0.26)	< 0.001
Sponge-stick (D)	-0.41 (-0.74/-0.08)	0.02	-0.48 (-0.71/-0.25)	< 0.001
Wet-dry double-swab (E)	-0.83 (-1.11/-0.55)	< 0.001	-0.58 (-0.80/-0.36)	< 0.001
	Time = 24 hours			
Destructive method (A)	0.00 (-)[3]	-	0.00 (-)[4]	-
Gauze cloth swab (B)	-0.02 (-0.19/0.16)	0.82	-0.02 (-0.10/0.12)*	0.84
Sponge swab (C)	-0.10 (-0.24/0.04)	0.16	-0.08 (0.18/0.01)*	0.09
Sponge-stick (D)	-0.09 (-0.23/0.05)	0.15	-0.07 (-0.17/0.02)*	0.15
Wet-dry double-swab (E)	-0.15 (-0.21/0.09)	0.01	-0.12 (-0.22/-0.03)*	0.01

[1]*Enterobacteriaceae* median recovered by destructive method after 2 h: 0.86 log CFU/cm^2 and [2]after 24 h: -0.40 log CFU/cm^2.
[3]*E. coli* median recovered by destructive method after 2 h: 0.52 log CFU/cm^2 and [4] after 24 h: -0.55 log CFU/cm^2.
*Estimated using linear regression, as median regression did not converge.

per cm^2 and "1" added in order to avoid negative numbers. Results below the limit of detection were set to 1 CFU per cm^2 for calculations.

Statistical analyses were conducted in Stata/MP 14.0 (StataCorp, College Station, TX), using ANOVA analysis and linear regression. Linear regression analyses were performed on the $\log_{10}$ transformed data with *Enterobacteriaceae* and *E. coli* as response variables in each model, and inoculum (pilot experiment only), sampling technique, and time of sampling as explanatory variables. All three regression methods produced results with a reasonable fit measured by the R^2 statistics (0.45–0.58). Model fit and residuals were checked using mainly graphical techniques. Residuals showed some deviating patterns, and to verify results a median regression (non-parametric regression) method was used to compare results to those obtained with the standard linear regression method. While coefficients changed with the more robust median regression, the conclusions drawn from the results would not have been different. Thus, we present only the quantile regression results *(Table 1 and Table 2)*. This paper focuses on relative performances and differences between the experimental variables, and since the results are not describing naturally contaminated carcasses but inoculated meat models in a laboratory study, the intercepts are not shown in the regression results.

RESULTS

The main purpose of the pilot experiment was to decide on the inoculation level for the main experiment. The pilot experiment demonstrated that most of the variance associated with *Enterobacteriaceae* recovery was linked to inoculum ($P < 0.001$), while sampling techniques ($P = 0.25$) and sampling

TABLE 2. Reduction in *Enterobacteriaceae* and *E. coli* in the confirmatory experiment by using median regression model. There were three sampling techniques (highest counts in the main study, A, B and D), two sampling times (2 and 24 h), and one inoculum level (10^4). The regression coefficients described the change in log_{10} CFU per cm^2 for either *Enterobacteriaceae* or *E. coli* as an explanatory variable changed by one unit, the other variables being held constant. Baseline levels for the categorical variables were sampling technique A and 2 h period between inoculation and sampling.

Variable	*Enterobacteriaceae* (log_{10})		*E. coli* (log_{10})	
	Coefficient (95% CI)	*P*-value	Coefficient (95% CI)	*P*-value
	Time = 2 hours			
Destructive method (A)	0.00 (-)[1]	-	0.00 (-)[2]	-
Gauze cloth swab (B)	-1.07 (-1.55/-0.58)	< 0.001	-0.29 (-0.39-0.19)	< 0.001
Sponge-stick (D)	-0.79 (-1.78/-0.20)	0.01	-0.19 (-0.42-0.04)	0.11
	Time = 24 hours			
Destructive method (A)	0.00 (-)[3]	-	0.00 (-)[4]	-
Gauze cloth swab (B)	-0.44 (-1.16/-0.28)	0.21	-0.15 (-0.36-.0.05)	0.14
Sponge-stick (D)	-0.44 (-1.11/-0.28)	0.21	-0.14 (-0.35-.0.05)	0.14

[1]*Enterobacteriaceae* median recovered by destructive method after 2 h: 0.56 log CFU/cm^2 and
[2]after 24 h: -0.16 log CFU/cm^2.
[3]*E. coli* median recovered by destructive method after 2 h: 0.08 log CFU/cm^2 and [4]) after 24 h:-0.70 log CFU/cm^2.

time ($P = 0.30$) were of lesser importance. Corresponding numbers for *E. coli* were for inoculum ($P < 0.001$), sampling technique ($P = 0.52$), and sampling time ($P = 0.25$). There were no significant differences between the results at 2 h and 12 h for either *Enterobacteriaceae* or *E. coli*, nor between 12 h and 24 h. Sampling at 12 h was therefore not included in the main experiment. No interaction effects were found. The initial inoculation level was 2.1 log CFU/cm^2 in the main study and 2.2 log CFU/cm^2 in the confirmatory study.

In the main study, the destructive sampling method (A) with a circular excision of 5 cm^2 area resulted in higher mean recovery of *Enterobacteriaceae* than the swabbing techniques, with the exception of the gauze cloth swab (B), both before and after chilling ($P < 0.05$ by ANOVA) *(Fig. 3)*. The numbers obtained using the destructive method (A) 2 h after inoculation was taken as the reference level *(Table 1 and Table 2)*. The coefficients in the regression equation quantify the expected change in *Enterobacteriaceae*/*E. coli* log per cm^2 from the changes in the explanatory variables; sampling techniques (B–E) and period after inoculation (24 h) *(Table 1 and Table 2)*. In the confirmatory study, the destructive sampling method (A) gave a higher recovery of *Enterobacteriaceae* than either the gauze cloth swab method (B) or the sponge-stick (D) method, both before and after chilling ($P < 0.05$ by ANOVA) *(Fig. 4)*. Swabbing before chilling (2 h after inoculation) with a gauze cloth (B) and sponge-stick (D) resulted in the second highest *Enterobacteriaceae* numbers in both the main study and confirmatory study, with a median that was 0.5 log/cm^2 lower than that of the destructive method. Sponge swab (C) resulted in a median value that was 0.7 log/cm^2 lower than that from excision (A), and the wet-dry double-swab technique (E) had 0.8 log/cm^2 lower median than excision (A) and its median was lower than that of all the other techniques ($P < 0.05$). The recovery of *Enterobacteriaceae* was lower for all techniques when sampling was carried out after chilling rather than before ($P < 0.05$). All the non-destructive methods had lower median recoveries of *Enterobacteriaceae* than the destructive method, by 0.1 – 0.2 log/cm^2 ($P < 0.05$).

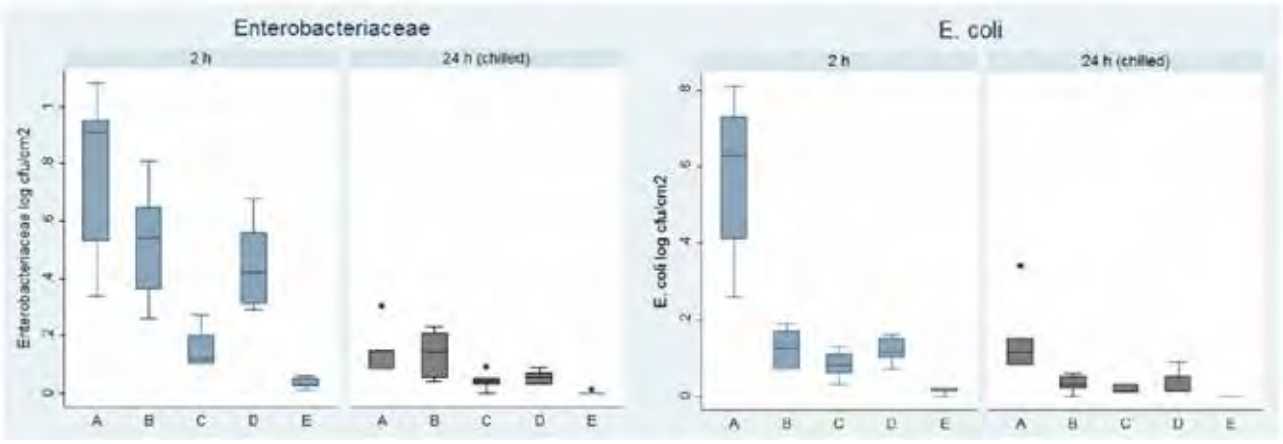

Figure 3. Results from the main experiment (n = 60), showing box-plots of the combinations of techniques and time sampled after inoculation. On each box, the central mark indicates the median, the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively, and the whiskers extend to the most extreme data points not considered outliers, the outliers are plotted individually using dot symbol. A: destructive method, B: gauze cloth swab, C: sponge swab, D: sponge-stick, E: wet-dry double swab; time 2 and 24 hours after inoculation. Results for Enterobacteriaceae *(left) and* E. coli *(right) measured as* log_{10} *CFU per* cm^2.

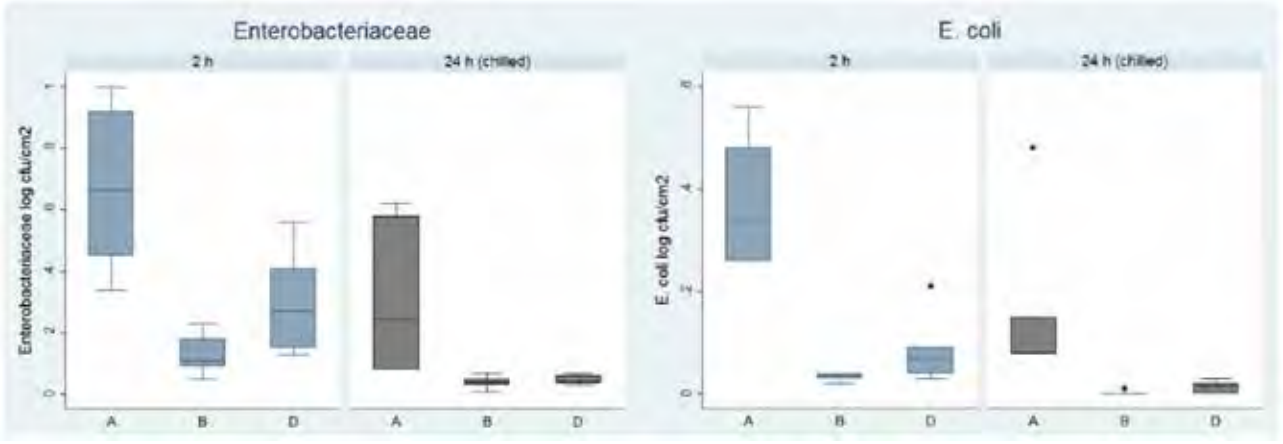

Figure 4. Results from the confirmatory experiment (n = 36), showing box-plots of the combinations of techniques and time sampled after inoculation. On each box, the central mark indicates the median, the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively, and the whiskers extend to the most extreme data points not considered outliers; the outliers are plotted individually using dot symbol A: destructive method, B: gauze cloth swab, D: Sponge-stick and time 2 and 24 hours after inoculation. Results for Enterobacteriaceae *(left) and* E. coli *(right) measured as* log_{10} *CFU per* cm^2.

The destructive sampling method (A) showed higher mean recovery of *E. coli* than the swabbing techniques ($P < 0.05$ by ANOVA) in both the main study and the confirmatory study, when sampling was performed both before (2 h) and after chilling (24 h after inoculation). In the main and confirmatory study, the median values for *E. coli* for all the swabbing techniques were 0.3–0.6 log/cm^2 lower than the median obtained using the destructive excision technique ($P < 0.05$) when sampling 2 h after inoculation. The effect of chilling was shown by the significant reduction in positive samples for *E. coli*. Before chilling, 97% (58/60) of the samples were positive (the gauze cloth technique (B) and wet-dry double-swab (E) had 1 negative each). After chilling at 4°C, 62% of the samples were positive (37/60): 50% in the group sampled using technique A, 66% in the group using technique B, 100% for technique C, 83 % using technique D, and 0% with technique E. The *E. coli* median values were also lower ($P < 0.05$) after chilling than without chilling for all sam-

pling techniques. When sampling was performed after chilling, the median values for all the swabbing techniques were 0.1–0.2 log/cm^2 lower than for the destructive method ($P < 0.05$). The efficacy of the sampling methods, in order according to recovery, were: destructive method > gauze cloth swab ≥ sponge-stick ≥ sponge swab > wet-dry double-swab.

In the main experiment, one inoculum level was used for all samples. As the results between the two sampling times (2 h and 24 h) differed in the main study, the regression results are presented separately for each sampling time *(Table 1)*. The effect of sampling technique was more marked for samples taken at 2 h.

Corresponding results for the confirmatory experiment, in which the three best techniques from the main experiment were used; destructive (A), gauze cloth swab (B), sponge-stick (C), are presented in *Fig. 4*, where two samples taken at 24 h yielded results below the detection limit for *Enterobacteriaceae* and *E. coli* for technique A. As for the main experiment, the statistical results for each sampling time are presented separately for the confirmatory study *(Table 2)*. The control samples (without inocula) in all experiments showed low contamination of the meat models (range of 0–36 CFU/cm^2 *Enterobacteriaceae* and *E. coli*).

DISCUSSION

The results confirm that the destructive sampling method, recommended by the EU *(17)* produces the highest recovery of *E. coli* from a specific, small area when 5 cm^2 of inoculated excised flank meat is tested, compared with four non-destructive swab sampling techniques. The difference between the recoveries obtained for *Enterobacteriaceae* using the destructive method and the gauze cloth swab was lower. This result is in accordance with other studies that have found higher numbers of total aerobic counts recovered by excision than swabbing *(13, 22, 24, 40)*. However, Gill and Jones found that swabbing with gauze and sponge retrieved numbers of coliforms and *E. coli* that were higher than those obtained by sampling by excision *(22)*. Our experiments further demonstrated differences between the non-destructive techniques used, with only two (B; gauze cloth swab and D; sponge-stick) of them assessed as being potentially appropriate for use in our subsequent baseline studies in a number of European abattoirs. Successful removal of bacteria from the surfaces of carcasses and onto the swabs depends on many factors associated with the carcass surfaces, such as moisture, temperature, hardness, and structure. Factors associated with the swab, including abrasiveness, swab type, pressure applied, moisture, number of strokes, area of swabbing, and operator-related differences, are also of relevance *(2, 7, 36)*. All these factors result in large variations among results. However, an important point is that swabbing covers larger areas than excision and may be more reliable for sampling carcasses with low numbers of bacteria that are spread unevenly over the carcass surface *(4)*. Lindblad et al. claimed that swabbing a larger (400 cm^2) with gauze pads met the same process hygiene criteria as excision of a small area (20 cm^2) *(30)*. Although efficacy of recovery of bacteria is important when selecting a sampling technique for carcasses, other factors also influence the choice. Swabbing is a quick, non-destructive method that is easy to use in commercial studies at operational chain speeds *(3)*. Swabbing is considered practical and cost efficient for the meat industry because it is less laborious than excision sampling and does not compromise meat quality.

Hutchison et al. sampled more than 1,700 carcasses during 70 separate visits to commercial abattoirs *(24)*. The results showed that variation in contamination was so large that calculation of a conversion factor between the different sampling techniques used on the natural contaminated carcasses was not possible *(24)*. Therefore, our comparative study was performed in a laboratory, using pre-defined inocula for contamination, in order to standardize the bacterial levels on the meat models. This approach was also intended to minimize confounding effects and ensure that bacterial recovery was sufficiently high that the performance of the different sampling techniques could be evaluated. The meat tissue model was artificially contaminated to mimic fecal contamination that may occur during the slaughtering process. The level of contamination was chosen in order to achieve quantifiable results from testing the model. The results from the pilot study showed that the high inoculum (10^6 CFU/mL) was unnecessarily high, while the low inoculum (10^3 CFU/mL) was too low, resulting in many samples below the detection limit. As inoculated meat models, rather than naturally contaminated carcasses were used, and since investigation of the differences between techniques was the main objective of the study, we have not presented the mean values. Although the inoculum size used in the main and the confirmatory study was rather high (10^4 CFU/mL), this was considered necessary to ensure bacterial recovery, as natural contamination levels of *E. coli* are normally very low, especially after carcass chilling. Furthermore, similar results have been observed from analysis of sheep carcasses in different European abattoirs (unpublished data).

The meat models used in our experiments were made using flanks from sheep, but the results are also valid for other meat species. Dorsa et al. evaluated sampling methods for recovery of bacteria inoculated onto beef carcass surfaces and found that tissue type (lean/adipose) did not alter the efficacy of the sampling methods *(13)*. Gallina et al. studied microbial recovery from naturally contaminated carcasses from several species and reported differences in recovery between samples from ruminants and horses *(19)*. This observation might be attributable to variations in the microbiota on carcasses from different species, the microorganisms' abilities to attach to the meat, the extent to which the different bacteria were stressed, and the need for bacterial resuscitation in the

analyses. In their study, Gallina et al. found that the excision method was superior to swabbing methods at recovering total viable counts from all species *(19)*. However, sponge swabbing on cattle, pigs and small ruminants resulted in microbial recoveries that were very similar to those obtained using the excision method *(19)*.

Bolton, like Hutchinson et al., comments that a quantitative conversion factor between excision and swabbing has not been established *(4, 24)*, probably due to the many sources of variation in swabbing data. Nevertheless, some studies have attempted to calculate the relationship in different ways. Edmonds claims that the recovery efficiencies of swabbing with different materials compared with excision vary widely, from 20% to 90% *(14)*, and Pepperell et al. states that values can even vary from 2 to 100% *(35)*. Ghafir and Daube found a recovery of 36% for *E. coli* for swabbing pig carcasses (600 cm^2) compared with the destructive method (20 cm^2), using log CFU/cm^2 in the calculation *(20)*. Gallina et al. *(19)* referred to the Italian ministry of Health *(38)*, which states that non-destructive methods recover 20% of the bacteria compared with the destructive method. The European microbiological criteria *(17)* define microbial limits of "m" and "M" for the destructive method, and m = 1.5/M = 2.5 log CFU/cm^2 *Enterobacteriaceae* is converted to m = 0.8/M = 1.8 log CFU/cm^2 for non-destructive techniques when calculating 20% of the CFU/cm^2 counts (note: not log-transformed units). Gallina et al. found the CFU recovered by swabbing was generally greater than 20% of the excision value, based on median results *(19)*. In our study, swabbing with gauze cloth corresponded to a recovery of 28% for *Enterobacteriaceae* counts (CFU/cm^2) compared with the destructive method, while the sponge stick had a recovery of 29%, sponge swab 8%, and wet-dry double swab 1%. All swab techniques had, on average, 17% of the recovery of the destructive method on warm meat samples. Although it is difficult to ascertain and standardize a conversion factor, the Italian conversion factor of 20% is similar to that observed with our results.

Growth of microorganisms during chilling is inhibited by the low temperatures and dry meat surfaces. Our results show that sampling warm meat provides higher recovery of bacteria, and thus we assume that sampling from warm carcasses provides a better overview of the slaughter hygiene at an abattoir than samples from chilled carcasses. Samples from chilled carcasses, however, may offer more information about the post-slaughter and chilling processes. These will vary between different slaughterhouses, depending on factors such as air velocity, temperature, and relative humidity *(5, 6)*. The US approach and the techniques used in Denmark and Norway involve sampling from chilled carcasses *(9, 10, 33)*. A major advantage of sampling chilled carcasses is that there are fewer time constraints, so the operator has more time to conduct the sampling appropriately. Blast chilling of pig carcasses is reported to reduce *E. coli* numbers by 1 log *(28)*. Ware et al. found that sampling by excision achieved a higher recovery after 24 h at 7°C than sampling with a sponge swab, because of bacterial attachment in the intervening period and therefore a reduction in the efficacy of swabbing *(39)*. In our study also, recovery using swabbing techniques, compared with that using the destructive method, was slightly reduced, from 17% to 14%, after chilling. This might be the effect of bacterial attachment to the meat.

Chilled carcasses are further along on the meat value chain towards the consumer than warm carcasses, and thus the results from sampling may be of greater public health significance. However, some abattoirs perform cutting and deboning of warm carcasses immediately after slaughter (hot boning), and sampling of chilled carcasses is therefore not possible. Investigations of chilled carcasses also require the use of analyses that take into account that *Enterobacteriaceae* and *E. coli* are stressed and need resuscitation. The use of non-selective medium (tryptone soya agar) in microbial analyses of these bacteria *(31, 32)* has been recommended for the recovery of stressed *Enterobacteriaceae (12, 27)*. One advantage of the NMKL125 method (Nordic Committee on Food Analysis) is the inclusion of a resuscitation step in non-selective medium for stressed or sub-lethally injured bacteria *(32)*. This, together with the low incubation temperature (37°C as opposed to 41.5 or 44°C) used for SimPlate, will also contribute to recovery of injured bacteria *(23)*.

The choice of hygiene indicator is another important consideration. A quantitative bacterial indicator to be used in a process monitoring method needs to provide results that can be enumerated for a considerable proportion of carcasses; at least 80% positive tests have been recommended *(21)*. Our experiments used high inocula to ensure that the results obtained could be interpreted, but this does not reflect the "real life" situation should these methods be used on routinely sampled cattle carcasses, where the contamination on many carcasses will be below the limit of detection. Thus, *E. coli* that is at a lower prevalence and concentration on carcasses will be a less suitable indicator for this purpose than *Enterobacteriaceae*.

Although the destructive method gives a higher recovery, swab sampling, especially using gauze cloth, is easier and quicker to use and has the highest recovery, after the destructive method. This sampling technique was therefore chosen as the method to be used for the subsequent baseline studies in European abattoirs.

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