Journal of Food Protection 86 (2023) 100155

Contents lists available at ScienceDirect

Journal of Food Protection

journal homepage: www.elsevier.com/locate/jfp

Research Paper

Assessment of ATP-Bioluminescence and Dipslide Sampling to Determine the Efficacy of Slaughterhouse Cleaning and Disinfection Compared with Total Aerobic and *Enterobacterales* Counts

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ARTICLE INFO

Keywords:
ATP
Dipslide
Enterobacterales
Indicator bacteria
Meat processing
Total aerobic bacteria

ABSTRACT

Inadequate cleaning and disinfection (C&D) in slaughterhouses can cause bacterial contamination of meat, resulting in foodborne disease and reduced meat quality. Different methods for monitoring the efficacy of C&D procedures are available, but few studies have assessed their reliability. This study examined C&D efficacy in slaughterhouses and evaluated the diagnostic performance of methods for measuring surface hygiene.

One red meat and one poultry slaughterhouse in Sweden were each visited on six occasions before and six occasions after C&D. Sampling points were sampled with: swabbing and plating for total aerobic bacteria (TAB) and *Enterobacterales* (EB); dipslides for total viable count; and ATP-bioluminescence tests. To evaluate the diagnostic performance of the dipslide and ATP-bioluminescence methods, the results were compared with (TAB) as a reference.

In total, 626 samples were collected. For the majority of samples, TAB was lower after than before C&D and EB were mainly detected before C&D, indicating C&D efficacy. Greater reductions in mean TAB were observed in processing areas (2.2 and 2.8 log CFU/100 cm^2 in red meat and poultry slaughterhouse, respectively) than in slaughter areas (1.3 log CFU/100 cm^2 in both slaughterhouses). Approximately half of all samples were assessed as non acceptably clean (52% for red meat and 46% for poultry slaughterhouse) according to previously published thresholds. Critical food contact surfaces that were insufficiently cleaned and disinfected were plucking fingers, shackles, and a post-dehairing table. Cleaning and disinfection of drains and floors were inadequate.

The ATP-bioluminescence method showed low specificity compared with the reference (TAB) in both the red meat (0.30) and poultry slaughterhouses (0.64). The sensitivity of dipslides was low (0.26) in the red meat slaughterhouse compared with TAB. A combination of ATP-bioluminescence and dipslides could provide more accurate estimates of C&D efficacy.

Food legislation within the European Union (EU) requires cleaning and disinfection (C&D) of surfaces in direct contact with food products (food contact surfaces, FCS) and non food contact surfaces (NFCS), including processing equipment on food premises (EC, 2004; Ninios et al., 2014). Sterilization is not achieved by C&D, so low microbial load can be expected on surfaces after C&D (Stanga, 2010). A threshold for satisfactory microbial hygiene on FCS or NFCS after C&D is not defined in EU legislation, meaning that food business operators (FBO) must decide their thresholds based on hazard analysis and critical control points (HACCPs), and good hygiene practices (GHPs) (Codex Alimentarius, 2020). A low microbial load minimizes the risk of cross-contamination, spread, and multiplication of pathogenic and food spoilage bacteria, that could have detrimental effects on public health and decrease the shelf-life of food. The slaughterhouse environment can easily be contaminated with organic debris such as fecal mat-

Abbreviations: C&D, Cleaning and disinfection; EB, *Enterobacterales*; TAB, Total aerobic bacteria.

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https://doi.org/10.1016/j.jfp.2023.100155
Received 15 June 2023; Accepted 28 August 2023
Available online 1 September 2023

ter and other body fluids. Therefore, C&D is an important hurdle in the control of foodborne pathogens and spoilage bacteria.

The active components in detergents used for C&D in red meat and poultry slaughterhouses are commonly alkaline compounds with or without chlorine (i.e., sodium hypochlorite, sodium hydroxide, potassium hydroxide), which are recommended to be used alternately with acidic compounds (i.e., peracetic acid, phosphoric acid, sulfuric acid). The active components in disinfectants are very similar to those in detergents (i.e., sodium hypochlorite, sodium hydroxide, alternated with peracetic acid, acetic acid, hydrogen peroxide). Instead of using alkaline or acidic agents as disinfectants, quaternary ammonium compounds are commonly used (García-Sánchez et al., 2017; Hutchison et al., 2007; Khamisse et al., 2012; Wang et al., 2018). When disinfectants are used in food production premises, the antimicrobial efficacy can be affected by various environmental factors such as temperature, humidity, surface materials, and residues of organic substances. However, standardized laboratory tests on the antimicrobial effect of disinfectants are usually performed in controlled conditions using suspension tests, and on surfaces that are easy to clean such as stainless steel, which is flat and rather resistant to scratching (SS-EN 14349:2012; SS-EN 1276:2019). Different types of surface materials are used in meat processing premises, and scratches and cracks that develop after a period of use can become harborage sites for bacteria (Fagerlund et al., 2017). These resident bacteria become part of the in-house microbiota, which is not removed by common C&D procedures and adds to continuous cross-contamination of food, thereby posing a threat to food safety and quality (Fagerlund et al., 2016; García-Sánchez et al., 2017). Moreover, pathogenic bacteria may persist on surfaces due to insufficient C&D, leading to outbreaks of foodborne diseases. For example, an outbreak with five-fold higher annual levels of campylobacteriosis occurred in Sweden in 2016–2017 (Lofstedt, 2019), caused by inadequate cleaning of chicken transport crates. *Listeria monocytogenes* is also well known to cause outbreaks due to its ability to form biofilm, survive in food production environments, and resist C&D procedures (Fagerlund et al., 2017; Stephan et al., 2015). Other important pathogens of concern in slaughter and carcass processing are *Salmonella enterica* and Shiga toxin-producing *E. coli* (STEC). *Salmonella enterica* has been recovered from cleaned and disinfected surfaces in swine and poultry slaughterhouses (Arguello et al., 2012; Marin et al., 2022), while STEC has been detected on surfaces in cattle slaughterhouses after C&D (Brusa et al., 2021; Tutenel et al., 2003).

A concern for slaughterhouse FBOs is the risk of cross-contamination of products by meat spoilage bacteria, i.e., *Pseudomonas* spp., *Acinetobacter* spp., *Stenotrophomonas* spp., and bacteria belonging to the order *Enterobacterales* (EB, a bacterial order which formerly only included family *Enterobacteriaceae*). Spoilage bacteria have been detected on surfaces such as conveyor belts and cutting tools after C&D (Maes et al., 2017, 2019; Møretrø et al., 2013; Møretrø & Langsrud 2017; Wang et al., 2018). There are indications that the microbial population of the slaughterhouse environment affect the microbial load on carcasses more than the indigenous microbiota of the slaughtered animal, and spoilage bacteria on meat have been traced back to contaminated surfaces in the slaughterhouse due to inadequate C&D (Peruzy et al., 2021; Samapundo et al., 2019).

Total aerobic bacteria (TAB) and EB can be used as indicators of the hygiene status in meat processing plants, while EB can be used as an indicator of fecal contamination in slaughterhouses (Althaus et al., 2017; Hutchison et al., 2007). A study in the UK found that almost one-third of 94 red meat slaughterhouses failed to meet the specified measured TAB threshold for acceptably clean surfaces (2 log CFU/cm^2) (Hutchison et al., 2007). Different methods for monitoring the efficacy of C&D procedures are available, including ATP-bioluminescence, contact plates (dipslides), and swabbing and plating of sponge/swab samples (Maes et al., 2017; Moore & Griffith, 2002; Møretrø et al., 2019). Methods such as ATP-bioluminescence and dipslides are easy to use compared with swabbing and plating. The EU standard (SS-EN ISO 18593:2018) for surface sampling does not specify sampling frequency and sampling points, which are therefore selected based on risk-based principles. Additionally, the standard mentioned above only describes two sampling methods, swabbing and dipslides, and not the ATP-bioluminescence method, which is widely used by the industry.

Several studies have investigated bacterial contamination on carcasses in slaughterhouses (Hansson et al., 2010; Hauge et al., 2023; Lindblad et al., 2006; Moazzami et al., 2021; Peruzy et al., 2021). However, there still seems to be a gap in knowledge concerning different hygiene indicator bacteria on different environmental surfaces and equipment. Additionally, there is a lack of published studies comparing different hygiene monitoring methods in industrial settings. Therefore, the aims of the present study were to determine the efficacy of C&D, and to evaluate the diagnostic performance of methods for assessing surface hygiene in slaughter areas and adjacent meat processing areas.

Materials and Methods

Study design. Two Swedish slaughterhouses were included in the study: a small/medium-scale red meat slaughterhouse, slaughtering approximately 100–120 swine and 25 cattle per day, and a large-scale poultry slaughterhouse, slaughtering approximately 220,000 broilers per day, both with adjacent areas processing raw meat (including cutting, meat preparation, and packaging facilities). Both slaughterhouses use a rotation of alkaline and acidic chemicals for C&D, and a low-pressure water pump (approximate pressure 28–35 bar) for their application during C&D (Fig. 1). Neither of the slaughterhouses uses forced ventilation to dry surfaces after C&D. Each slaughterhouse was visited on six occasions before C&D, and on six occasions after C&D. All visits before C&D were made after the end of the last working shift, which was immediately before C&D (Monday afternoon/evening), and all the visits after C&D were made before the start of the morning shift (Tuesday morning). Sampling was carried out from October 2020 to October 2021.

Identification of sampling points. The quality assurance staff at the slaughterhouses were involved in the selection of sampling points. When practically possible, surfaces known to be difficult to clean and/or critical due to potential cross-contamination of the meat were selected as sampling points. Both FCS and NFCS, including scald water, were selected for sampling, with 11 sampling points in the red meat slaughterhouse (6 in the slaughter area, 5 in the processing area) and 10 sampling points in the poultry slaughterhouse (5 in the slaughter area, 5 in the processing area). In the red meat slaughterhouse, cattle and swine were slaughtered in the same slaughter area, while only beef was handled in the processing area. All sampling points were sampled on each sampling occasion, before and after C&D procedures.

Sampling procedure and sample analysis. On each sampling occasion, each sampling point, except scald water, was sampled with three different methods: swabbing with sponge (Hydra-Sponge 1.5*3 inches Sponge w/10 mL Letheen broth, 3M Health Care, St. Paul, USA)/swab (Swab-sampler with 10 mL D/E Neutralizing broth, 3M Health Care, St. Paul, USA), dipslide (Envirocheck® Contact TVC, Merck KGaA, Darmstadt, Germany), and ATP-bioluminescence tests (Surface ATP/Water-Free ATP, Clean-Trace™ Test, 3M Health Care, St. Paul, USA), on surfaces adjacent to each other (Fig. 2). Sampling was performed aseptically. When possible on flat surfaces, sterilized stainless steel frames were used to delineate an exact sampling area. For practical reasons, different size frames were used for swabbing with sponge/swab (100 cm^2) and ATP-bioluminescence (25 cm^2). For sampling points with smaller areas, 25 cm^2 were swabbed with sponge/swab. Five plucking fingers, one shackle, and five salt injector needles were sampled on each occasion, and the total sampling area

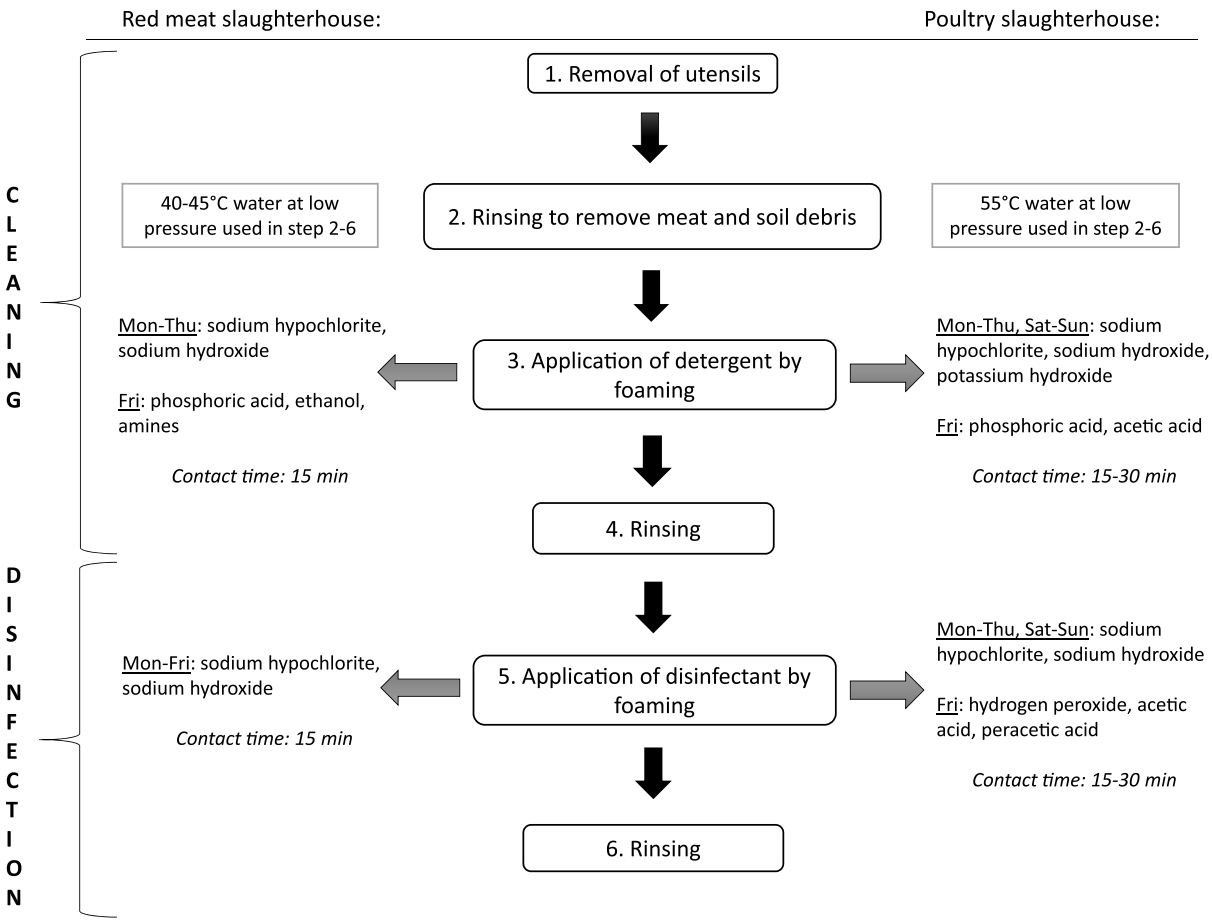

Figure 1. Flow chart of the general cleaning and disinfection procedures at the two slaughterhouses included in this study.

was estimated to be 100 cm^2, 25 cm^2, and 25 cm^2, respectively). The area of the dipslides was always 19 cm^2. The same individuals performed all samplings during the study. The temperature of the scald water was measured on one sampling occasion in each slaughterhouse.

After each sampling, the sponges, swabs, dipslides, and scald water were transported in an insulated box with refrigerant gel packs to the Biomedical Sciences and Veterinary Public Health laboratory at the Swedish University of Agricultural Sciences, Uppsala, Sweden. The temperature was checked upon arrival. Only samples with temperature 2–8°C were accepted for analysis, which began within 12 h after sampling.

Swabbing and plating. The prehydrated sponges (7.6 by 4 cm) were used for swabbing and enumeration of TAB and EB (Fig. 2A). For practical reasons, cutting blades and salt injector needles were sampled using a prehydrated swab sampler (Fig. 2B). Swabbing was performed using firm and even pressure, with overlapping horizontal and vertical strokes. Approximately 45 mL of scald water was collected in a sterile plastic bottle from the upper part of the scald water tank, before C&D (directly after slaughter finished) and after C&D (immediately before the next slaughter shift started).

In the laboratory, sponges were homogenized for 120 s at 240 rpm (easyMIX Lab Blender, AES-Chemunex, Weber Scientific, Hamilton, New Jersey, USA), while swabs and scald water were vortexed for approximately 10 s. From each sponge/swab/scald water sample, a 10-fold serial dilution in 0.1% (v/v) peptone water (Dilucups, LabRobot Products AB, Stenungsund, Sweden) was prepared. TAB were enumerated according to NMKL 86 (5th Ed. 2013). From the dilution series prepared for each sample, 1.0 mL aliquots of each dilution were plated on an aerobic count plate (3M Petrifilm™, 3M Health Care, St. Paul, USA) and left to solidify. Plates were then incubated at 30 ± 1 °C for 72 ± 3 h. Bacterial counts were preferably performed on plates with 25–250 colonies and expressed as log CFU/100 cm^2. The detection limit was 1.0 log CFU/area sampled, or for scald water 1.0 log CFU/mL.

Analysis of EB was performed according to NMKL 144 (3rd Ed. 2005). The previously prepared 10-fold dilutions were used to estimate EB counts in the samples. From each dilution, 1.0 mL was plated on an *Enterobacteriacae* count plate (3M Petrifilm™, 3M Health Care, St. Paul, USA) and left to solidify. Plates were then incubated at 37 ± 1 °C for 24 ± 2 h. Bacterial counts were preferably performed on plates with 15–150 colonies, and the number of EB was expressed as log CFU/100 cm^2. The detection limit was 1.0 log CFU/area sampled, or for scald water 1.0 log CFU/mL.

Dipslide method. The dipslide test for total viable count (TVC) was pressed firmly and evenly onto the surface to be sampled, and then turned over and the second side was pressed in the same way next to the first sampling site (Fig. 2C). After transport to the laboratory, the dipslides were incubated in upright position at 37 ± 1 °C and checked for growth after 48 ± 4 h. Colonies on both sides of the dipslide (19 cm^2) were counted, and the TVC was expressed as log CFU/100 cm^2.

ATP-bioluminescence method. To determine the level of cellular material on surfaces, adenosine triphosphate (ATP)-bioluminescence was used. The ATP level in scald water was measured with Water-Free ATP tests. Before use, Surface ATP and Water-Free ATP tests were kept in foil pouches to protect the ATP reagent from light and stored at 2–8°C. Approximately 24 h before sampling, they were moved to room temperature (around 20°C) according to the manufacturer's instruc-

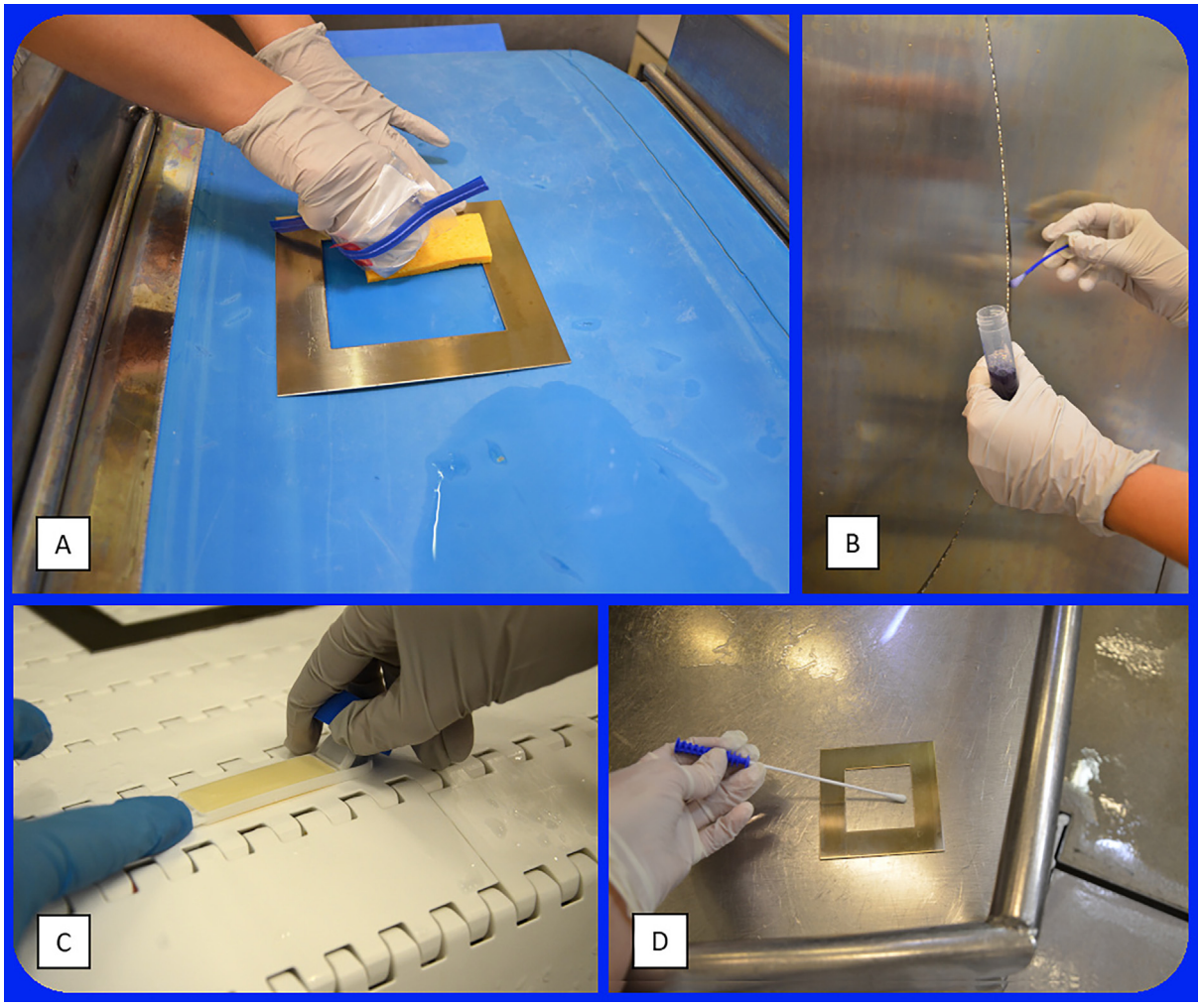

Figure 2. A) Swabbing conveyor belt with prehydrated sponge, B) swabbing cutting blade with prehydrated swab, C) pressing dipslide on conveyor belt and, D) swabbing post-dehairing table with ATP-swab.

Table 1
Selected thresholds for clean surfaces regarding total aerobic bacteria (TAB), total viable count (TVC), ATP-bioluminescence (relative light units, RLU), and *Enterobacterales* (EB)

	Selected threshold/ cm^2	Source
TAB - swabbing & plating	2.5 CFU	Ching et al. (2021); Griffith, 2005; Ninios et al. (2014)
TVC- dipslides	1.0 CFU	Eurofins Food & Feed Testing Sweden AB (2021), based on Swedish Food Agency (1998)
ATP - bioluminescence	1.5 RLU	Technical bulletin 3M™ Clean-Trace ™ Hygiene Monitoring and Management System (2019)
EB - swabbing & plating	1.0 CFU	Gómez et al. (2012); Statutory Instruments (2002). The meat (Hazard Analysis Critical Control Point) (England) Regulations 2002

tions. Each surface was swabbed with firm and even pressure, using overlapping horizontal and vertical strokes, and at the same time, the swab was rotated over its own axis (Fig. 2D). Water ATP swabs used to measure the ATP levels in scald water were immersed completely under the liquid surface and shaken gently to remove possible air bubbles. ATP levels were measured within 2 h of sampling by placing the swabs into the ATP monitoring device (Clean-Trace LM1, 3M Health Care, St. Paul, USA). Results were recorded as relative light units (RLU) within the system device range of 0–6.0 log RLU. Surface ATP test results were expressed as log RLU/100 cm^2 and Water-Free ATP test results as log RLU/145 μL ± 15 μL (the volume of liquid tested).

Thresholds for clean surfaces. To assess whether the estimated number of bacteria or organic residues remaining on a surface after C&D was acceptable, thresholds were selected for each sampling method/bacterial group (Table 1). Due to lack of thresholds for clean surfaces in current legislation, selected values were first chosen from instructions from producers of the sampling materials used in the study (3M Science Applied to Life, 2019; Eurofins Food & Feed Testing Sweden AB, 2021). If such instructions were lacking, thresh-

olds from the literature and in proposed legislation were used (Ching et al., 2021; Gómez et al., 2012; Griffith, 2005; Ninios et al., 2014; Statutory Instrument, 2002; Swedish Food Agency, 1998). The thresholds used routinely in the two slaughterhouses were also considered.

Statistical analysis. To evaluate the reduction in bacterial and ATP levels before and after C&D, R Studio software (RStudio version 1.2.1335) was used. To enable comparison of the methods, the results were transformed into CFU/RLU per 100 cm^2. The values were log_{10}-transformed and modeled using Anova. The factors sampling point, occasion, and before/after were fixed factors in the model. Post hoc tests were performed to determine significantly different mean levels of bacteria before and after C&D for different sampling points, using Tukey's adjustment. Residuals were checked to confirm that they fulfilled the assumption of normal distribution and equal variances. Differences between mean values before and after C&D were deemed significant at $P < 0.05$.

To evaluate the diagnostic performance of the dipslide and ATP-bioluminescence methods, swabbing and plating for cultivation of TAB was used as a reference method. Results were only included in the comparison of methods when all three sampling methods were successfully conducted at the same sampling point on the same sampling occasion. The sensitivity and specificity calculations were according to Bonita et al. (2006).

Values were considered *true positives* when results from the reference method and the dipslide/ATP-bioluminescence methods indicated non acceptable level of cleanliness. Values were considered *true negatives* when results from the reference method and the dipslide/ATP-bioluminescence methods were considered to indicate acceptable cleanliness. Values were considered *false positives* when acceptably clean according to the reference method but non acceptable according to the dipslide/ATP-bioluminescence methods. Finally, values were considered *false negatives* when not acceptably clean according to the reference method, but acceptable according to the dipslide/ATP-bioluminescence methods. Indicators of diagnostic performance (accuracy, Ac; sensitivity, Se; specificity, Sp; positive predictive value, PPV; negative predictive value, NPV; Cohen's kappa agreement coefficient, κ) were calculated using the statistical software MedCalc Software Ltd (Ostend, Belgium). For Cohen's kappa coefficient, a value of $\kappa > 0.9$ indicates almost perfect agreement, values between 0.8 and 0.9 indicate strong agreement, from 0.6 to 0.79 moderate agreement, from 0.4 to 0.59 weak agreement, from 0.21 to 0.39 minimal agreement, and from 0 to 0.2 no agreement (McHugh, 2012).

Results

In total, 626 samples were collected before ($n = 313$) and after ($n = 313$) C&D procedures (these samples included scald water, the results for which are presented separately). All sampling points could not be sampled on all occasions, (e.g., when the cleaning staff started to clean earlier than planned). Each sampling point was sampled on 4–6 occasions before and 4–6 occasions after C&D. All samples had a temperature of 2–8°C on arriving at the laboratory and were accepted for analysis. In both slaughterhouses, most surfaces were visually clean after C&D, but traces of feces, feathers, meat, fat, etc. were observed on some surfaces. The majority of the surfaces were wet at the time of sampling, especially after C&D.

Total aerobic bacteria. In the red meat slaughterhouse, TAB could be enumerated from 98% (57/58) of the samples taken before C&D and 90% (52/58) of the samples taken after C&D. For the majority (90%) of the samples after C&D, the bacterial numbers were lower than in the corresponding samples before C&D. However, 10% (6/58) of the samples had TAB levels 0.1–2 log higher after C&D, representing samples from the post-dehairing table, the drain in the processing area, and the soft conveyor belt (3/6, 2/6, 1/6 samples, respectively). The greatest mean reduction (>3.0 log CFU/100 cm^2) was recorded for the cutting board. Overall, for 52% (30/58) of the samples taken after C&D from the red meat slaughterhouse, the values were above the selected threshold for clean samples (2.5 CFU/cm^2, equal to 2.4 log CFU/100 cm^2) (Table 1). For FCS, the values were above this threshold for 40% (14/35) of the samples. The highest mean values after C&D were observed for the drains, the post-dehairing table, and the conveyor belt for pig organs, with the highest individual bacterial count in one sample from the post-dehairing table (6.9 log CFU/100 cm^2). The table for cattle organs had the lowest mean value after C&D (Table 2).

In the poultry slaughterhouse, TAB could be enumerated from all samples (48/48) taken before C&D and 73% (35/48) of samples taken after C&D. For the majority (92%) of the samples, the bacterial numbers were lower after C&D than in the corresponding samples before C&D, but for 8% (4/48) of the samples, the values were 0.1–1.3 log higher after C&D. These samples were from the shackles and lairage floor (3/5 and 1/5 samples, respectively). The strongest C&D effect was seen for the salt injector needles (>3.0 log reduction). Overall, 46% (22/48) of the samples from the poultry slaughterhouse were non acceptably clean (>2.4 log CFU/100 cm^2). All samples of the plucking fingers and shackles were non acceptably clean after C&D. For FCS, 35% (13/37) were assessed as non acceptably clean. The highest mean values after C&D were observed for the plucking fingers, shackles, lairage floor, and drain, where one sample from the lairage floor had the highest individual bacterial count (7.0 log CFU/100 cm^2). The lowest mean values after C&D were observed for conveyor belts, cutting blade, and salt injector needles (Table 2).

In terms of C&D efficacy at the two slaughterhouses, the reduction in mean TAB at the red meat slaughterhouse was 1.3 and 2.2 log CFU/100 cm^2 in the slaughter and processing area, respectively. The corresponding reductions in the poultry slaughterhouse were 1.3 and 2.8 log CFU/100 cm^2, respectively. Moreover, the conveyor belts in the poultry slaughterhouse had lower bacterial numbers after C&D than the conveyor belts in the red meat slaughterhouse. A greater reduction after C&D was observed in the processing areas, except the drains, and the processing areas had more acceptably clean samples compared with the slaughter areas in both slaughterhouses. In the red meat slaughterhouse, the conveyor belts located in the processing area had a higher number of acceptably clean samples and greater TAB reductions than the conveyor belt located in the slaughter area. In the poultry slaughterhouse, TAB could be enumerated after C&D on the cutting blade in the slaughter area in 4/5 samples, but on the cutting blade in the processing area in only 2/6 samples. Furthermore, the mean TAB count after C&D was higher for the cutting blade in the slaughter area than for the cutting blade in the processing area (1.6 and 0.8 log CFU/100 cm^2, respectively), even though these sampling points were very similar and the same C&D procedure was used. The drains (in both slaughter and processing areas) and lairage floor were non acceptably clean on most sampling occasions in both slaughterhouses. The TAB reduction seen for two drains, one in the red meat slaughterhouse (sampling point 5) and one in the poultry slaughterhouse (sampling point 21), was 2.4 log and 2.3 log, respectively, which were among the greatest reductions observed in this study. However, since the mean TAB values before C&D were very high (5.9–6.0 log CFU/100 cm^2), high bacterial levels still remained after the C&D procedure (Table 2).

Total viable count-Dipslide. In the red meat slaughterhouse, TVC could be enumerated from 96% (52/54) of the dipslides before C&D and from 35% (19/54) of the dipslides after C&D. A total reduction in TVC on all dipslides was observed for the conveyor belts and the trolley in the processing area. In 7% (4/54) of the dipslides, the values were 0.2–1.3 log higher after C&D than on the corresponding dipslides before C&D, representing dipslides from the post-dehairing table and the cutting blade for carcasses (3/5, and 1/5 dipslides respectively). In the slaughter area, the post-dehairing table, the drain, and the cutting blade were considered nonacceptably clean (4/5, 1/5, and

Table 2
Mean log CFU/100 cm^2 of total aerobic bacteria (determined by swabbing and plating), log CFU/100 cm^2 of total viable count (on dipslides) and log RLU/100 cm^2 of ATP-bioluminescence before and after cleaning and disinfection (C&D), mean reduction after C&D and percentage of acceptably clean samples according to selected thresholds. Scald water bacterial count was measured as log CFU/mL for TAB and TVC, and as RLU/145 μL for ATP-bioluminescence

Slaughterhouse	Area	Sampling point		Total aerobic bacteria				Total viable count, dipslides				ATP-bioluminescence			
				Mean before C&D	Mean after C&D	Mean reduction	% acceptable samples	Mean before C&D	Mean after C&D	Mean reduction	% acceptable samples	Mean before C&D	Mean after C&D	Mean reduction	% acceptable samples
Red meat	**Slaughter**	**1**	Post-dehairing table pigs, stainless steel (upper part)[b]	4.0 ± 0.6	4.0 ± 1.7	0.001 ± 1.1	17	2.9 ± 0.5	2.3 ± 1.3	0.6 ± 1.3	20	3.9 ± 0.3	2.4 ± 0.2	1.5* ± 0.4	20
		2	Scald water[b]	4.7 ± 1.3	1.8 ± 1.0	2.8* ± 1.4	[a]	[a]	[a]	[a]	[a]	3.8 ± 0.5	2.1 ± 0.3	1.8* ± 0.8	[a]
		3	Table for cattle organs, stainless steel (upper part)[c]	3.0 ± 0.7	1.3 ± 0.9	1.7* ± 1.1	100	2.2 ± 1.3	1.0 ± 0.9	1.3* ± 1.3	100	3.7 ± 0.5	2.3 ± 0.2	1.4* ± 0.6	20
		4	Conveyor belt pig organs, soft plastic (upper part)[c]	4.6 ± 0.7	3.0 ± 1.0	1.6* ± 2.1	17	2.9 ± 0.6	0.6 ± 0.9	2.3* ± 1.1	100	4.4 ± 0.4	2.9 ± 0.3	1.6* ± 0.5	0
		5	Drain, stainless steel (inside and outside)[c]	6.0 ± 0.8	3.6 ± 1.0	2.4* ± 0.4	17	3.4 ± 0.5	0.8 ± 1.1	2.7* ± 1.4	80	4.9 ± 0.4	3.6 ± 0.5	1.3* ± 0.6	0
		6	Cutting blade cattle/pig carcasses, stainless steel[b]	2.2 ± 1.5	1.5 ± 1.6	0.7 ± 1.9	80	2.4 ± 1.4	1.6 ± 1.0	0.8* ± 1.7	60	4.9 ± 0.5	3.9 ± 0.7	1.0* ± 0.9	0
	Processing	**7**	Cutting board, plastic (upper part)[b]	5.2 ± 0.5	2.0 ± 0.9	3.2* ± 0.8	50	2.9 ± 0.4	0.4 ± 0.6	2.5* ± 0.7	100	5.5 ± 0.5	3.0 ± 0.3	2.5* ± 0.5	0
		8	Conveyor belt, soft plastic (upper part)[b]	4.2 ± 1.0	1.7 ± 1.5	2.5* ± 2.5	67	1.9 ± 0.3	0.0 ± 0.0	1.9* ± 0.3	100	5.0 ± 0.5	2.7 ± 0.5	2.3* ± 0.9	0
		9	Conveyor belt, hard plastic (upper part)[b]	4.7 ± 0.5	2.0 ± 0.8	2.7* ± 1.1	83	2.6 ± 0.4	0.0 ± 0.0	2.6* ± 0.4	100	5.5 ± 0.4	2.4 ± 0.3	3.1* ± 0.6	40
		10	Trolley, stainless steel (bottom)[b]	4.2 ± 0.9	1.5 ± 1.1	2.7* ± 1.4	67	2.4 ± 0.5	0.0 ± 0.0	2.4* ± 0.5	100	5.4 ± 1.2	1.8 ± 0.4	3.6* ± 1.0	100
		11	Drain, stainless steel (inside and outside)[c]	4.8 ± 0.5	4.6 ± 0.4	0.1 ± 0.8	0	2.7 ± 1.0	0.5 ± 0.7	2.1* ± 0.6	100	4.2 ± 0.7	3.5 ± 0.2	0.7* ± 0.8	0
Poultry	**Slaughter**	**12**	Cutting blade bleeding, stainless steel[b]	4.1 ± 0.9	1.6 ± 1.1	2.5* ± 0.7	80	3.8 ± 0.2	2.9 ± 0.6	0.9* ± 0.6	0	3.3 ± 0.3	3.0 ± 0.8	0.4 ± 1.0	0
		13	Scald water[b]	5.4 ± 0.1	2.4 ± 1.5	3.0* ± 1.5	[a]	[a]	[a]	[a]	[a]	5.1 ± 0.5	2.6 ± 1.0	2.5* ± 1.2	[a]
		14	Plucking fingers, rubber[b]	7.1 ± 0.8	5.5 ± 1.6	1.6 ± 1.0	0	3.6 ± 0.2	2.8 ± 0.6	0.8* ± 0.6	0	5.2 ± 1.2	4.7 ± 1.0	0.5 ± 1.0	0
		15	Shackle after stunning, stainless steel[b]	5.6 ± 0.9	5.2 ± 0.8	0.3 ± 1.4	0	3.5 ± 0.4	2.7 ± 1.5	0.9* ± 1.5	20	3.6 ± 0.9	3.3 ± 0.6	0.3 ± 1.1	0
		16	Floor lairage, concrete[c]	7.1 ± 0.2	6.5 ± 0.5	0.6 ± 0.5	0	3.9 ± 0.1	3.7 ± 0.2	0.2 ± 0.2	0	4.3 ± 0.3	3.8 ± 0.6	0.5 ± 0.7	0
	Processing	**17**	Conveyor belt, soft plastic (upper part)[b]	3.9 ± 0.7	1.1 ± 0.6	2.8* ± 0.6	100	2.9 ± 0.3	0.3 ± 0.6	2.6* ± 0.7	100	4.6 ± 0.3	2.0 ± 0.3	2.6* ± 0.5	75
		18	Conveyor belt, hard plastic (upper part)[b]	3.3 ± 0.6	0.6 ± 0.7	2.7* ± 0.8	100	2.3 ± 1.1	0.2 ± 0.4	2.1* ± 1.5	100	4.7 ± 0.2	1.9 ± 0.3	2.8* ± 0.4	80
		19	Cutting blade thighs, stainless steel[b]	3.1 ± 0.2	0.8 ± 1.3	2.4* ± 1.1	83	3.5 ± 0.2	0.0 ± 0.0	3.5* ± 0.2	100	4.9 ± 0.5	1.7 ± 0.3	3.2* ± 0.5	80
		20	Salt injector needles, stainless steel[b] [d]	4.4 ± 0.9	0.7 ± 1.5	3.7* ± 1.0	80	3.3 ± 0.3	0.8 ± 0.9	2.6* ± 0.6	100	3.7 ± 0.3	1.2 ± 0.8	2.5* ± 1.1	100
		21	Drain, stainless steel (inside and outside)[c]	5.9 ± 0.7	3.7 ± 1.6	2.3* ± 1.2	33	3.6 ± 0.1	1.4 ± 1.1	2.2* ± 1.0	60	4.2 ± 0.5	3.4 ± 0.5	0.8 ± 0.4	0

[a]Not applicable. [b]Food contact surface. [c]Non food contact surface. [d]Hard plastic under needles was sampled for dipslides. ± Standard deviation. *Significant reduction at $P < 0.05$.

2/5 dipslides, respectively) (Table 2). All sampling points in the processing area were considered acceptably clean after C&D, according to the selected threshold (1.0 CFU/cm^2, equal to 2.0 log CFU/100 cm^2) (Table 1).

In the poultry slaughterhouse, TVC could be enumerated from 98% (44/45) of the dipslides before C&D and from 60% (27/45) of the dipslides after C&D. A complete reduction in TVC was observed on the cutting blade for thighs on all dipslides. In 4% (2/45) of the dipslides, which were from the shackles and the hard conveyor belt, the values were 0.5 log and 1.0 log higher respectively after C&D than on the corresponding dipslides before C&D. The lairage floor had the highest mean values after C&D and the lowest reduction. Almost half of the samples (47%, 21/45) were considered non acceptably clean after C&D. The cutting blade for bleeding, plucking fingers, shackles, and lairage floor had the fewest acceptably clean samples and showed limited effect of C&D (<1.0 log reductions) (Table 2).

ATP-bioluminescence method. In the red meat slaughterhouse, RLU values >0 were obtained for all samples before C&D (50/50) and after C&D (50/50). Only one sample (drain in the processing area) had a higher RLU value (0.2 log higher) after C&D than in the corresponding sample before C&D. The greatest mean RLU reductions (>3.0 log) were observed for the hard conveyor belt and the trolley, both located in the processing area. The highest individual value after C&D was obtained for one sample from the cutting blade for carcasses (4.8 log RLU/100 cm^2). The majority (82%) of the samples were considered non acceptably clean according to the selected threshold (1.5 RLU/cm^2, equal to 2.2 log RLU/100 cm^2) (Table 1). Higher mean RLU values were observed in the processing than in the slaughter area before C&D, but the reduction was greater in the processing area, resulting in similar RLU levels after C&D in both areas. The trolley was the only sampling point that was acceptably clean on all sampling occasions (Table 2).

In the poultry slaughterhouse, RLU values >0 were obtained for all samples before C&D (43/43) and for 98% (42/43) of the samples after C&D. In 9% (4/43) of the samples, the values were 0.2–1.5 log higher after C&D than in the corresponding samples before C&D. More than half of the samples (65%, 28/43) were considered non acceptably clean after C&D. The highest mean RLU values after C&D were observed for the lairage floor and plucking fingers, with the latter having the highest individual value in one sample (5.6 log RLU/100 cm^2). The only sampling point which was considered acceptably clean on all sampling occasions was the salt injector needles. Other sampling points with low mean RLU values after C&D were the hard conveyor belt and the cutting blade for thighs in the processing area (<2 log RLU/100 cm^2) (Table 2).

Diagnostic performance of dipslide and ATP-bioluminescence methods. In general, method accuracy, measured as agreement of the dipslide and ATP-bioluminescence results with the reference method (TAB), was higher for the poultry slaughterhouse than for the red meat slaughterhouse. In the red meat slaughterhouse, sensitivity was very low (Se = 0.26) for the dipslide method, with 17 dipslides from six different sampling points assessed as acceptable according to the dipslide method, while the reference method assessed the level of cleanliness at those points as non acceptable, indicating a high number of false negatives. The ATP-bioluminescence method showed low specificity in the red meat slaughterhouse (Sp = 0.30), in which 19 samples from seven different sampling points were assessed as non acceptable according to the ATP-bioluminescence results while the reference method assessed the level of cleanliness as acceptable. Cohen's kappa (κ) values indicated minimal level of agreement with the reference method for both the dipslide and ATP-bioluminescence methods in the red meat slaughterhouse. In the poultry slaughterhouse, the κ values indicated that the dipslide method had moderate agreement and the ATP-bioluminescence had weak agreement with the reference method (Table 3).

Enterobacterales. In the red meat slaughterhouse, EB could be enumerated from 66% (38/58) of the samples before C&D, with a mean count of 2.3 ± 0.5 log CFU/100 cm^2. After C&D, EB could only be enumerated from 7% (4/58) of the samples, all from the drains (1.6, 2.2, 2.2, 3.4 log CFU/100 cm^2). The selected threshold for clean samples (1.0 CFU/cm^2, equal to 2.0 log CFU/100 cm^2) (Table 1) was exceeded in 5% (3/58) of the samples.

In the poultry slaughterhouse, EB could be enumerated from 88% (42/48) of the samples before C&D, with a mean count of 2.6 ± 0.5 log CFU/100 cm^2. After C&D, EB could be enumerated from 25% (12/48) of the samples, representing plucking fingers, shackles, lairage floor, and drain (3/5, 3/5, 4/5, 2/6 samples, respectively). The selected threshold for clean samples was exceeded in 19% (9/48) of the samples. The highest EB values (>3.0 log CFU/100 cm^2) were observed in three samples (shackles and lairage floor).

Scald water. Before C&D, all scald water samples were visibly dirty and had a strong smell. The mean TAB values before C&D were similar, and the reductions in TAB and RLU were significant in both slaughterhouses (Table 2).

In the red meat slaughterhouse, four scald water samples were analyzed before C&D and four samples after C&D. In all samples (8/8), TAB and RLU values were above the detection limit, before and after C&D. After C&D, two samples had TAB values >2.0 log CFU/mL and one sample had a RLU value >2.0 log RLU/145 μL. *Enterobacterales* were detected in one of the samples before C&D, but not detected in any of the samples after C&D. The temperature of scald water was measured on one sampling occasion and was 60.3°C directly after slaughter/before C&D and 44.5°C after C&D.

In the poultry slaughterhouse, five scald water samples were analyzed before C&D and five samples after C&D. Total aerobic bacteria could be enumerated from all samples before C&D, and from four samples after C&D. RLU values >0 were obtained for all samples (before and after C&D, 10/10), and three samples after C&D had RLU values >3.0 log RLU/145 μL. EB could be enumerated from all samples before C&D (mean 2.5 ± 0.9 log CFU/mL), but not from any of the samples after C&D. The temperature of scald water was measured on one sampling occasion and was 53.7°C directly after slaughter/before C&D and 44.3°C after C&D.

Discussion

Most surfaces sampled in this study were visibly clean after C&D. However, this did not mean that bacteria were absent, which is in agreement with previous findings (Khamisse et al., 2012). Moreover, visible dirt was observed after C&D on some sampling points such as plucking fingers and shackles, the uneven surfaces of which appear difficult to clean. These sampling points were considered non acceptably clean and EB were detected in the majority of the samples after C&D. This is consistent with previous findings that cleaned shackles and plucking fingers are among the most contaminated surfaces in poultry slaughterhouses (García-Sánchez et al., 2017; Zeng et al., 2021). In the poultry slaughterhouse examined in the present study, there was no standard operating procedure (SOP) for cleaning and disinfecting the shackles. They were close to other equipment that was cleaned and disinfected, and thus were only cleaned unintentionally *in situ* (without removal from the overhead conveyor system). Cleaning of shackles *in situ* has previously been observed in another slaughterhouse (Samapundo et al., 2019). This could explain the inadequate cleaning of the shackles, which were dirtier after C&D than before on more than half of the sampling occasions. The plucking fingers were included in the SOP, but the quality assurance staff reported difficulties in cleaning this type of irregular rubber surface. Moreover, the slaughterhouse did not include sampling of plucking fingers and shackles in its hygiene monitoring protocol, because these surfaces belong to the slaughter area, which is considered a "dirty" area of the slaughterhouse. In gen-

Table 3
Diagnostic accuracy of the dipslide and ATP-bioluminescence methods compared with swabbing and plating for total aerobic bacteria (TAB) as the reference method. Values in brackets indicate 95% confidence interval

Slaughterhouse	Red meat		Poultry	
	TVC-dipslide	ATP-bioluminescence	TVC-dipslide	ATP-bioluminescence
Total No. of samples	50	50	43	43
No. of nonacceptably clean samples	7	41	21	28
No. of nonacceptably clean samples with TAB	23		21	
Accuracy	0.64 (0.49–0.77)	0.60 (0.45–0.74)	0.81 (0.67–0.92)	0.79 (0.64–0.90)
Sensitivity	0.26 (0.13–0.47)	0.96 (0.79–0.99)	0.81 (0.60–0.92)	0.95 (0.77–0.99)
Specificity	0.96 (0.82–0.99)	0.30 (0.16–0.49)	0.82 (0.62–0.93)	0.64 (0.43–0.80)
Positive predictive value	0.86 (0.49–0.97)	0.54 (0.39–0.68)	0.81 (0.60–0.92)	0.71 (0.53–0.85)
Negative predictive value	0.60 (0.46–0.74)	0.89 (0.57–0.98)	0.82 (0.62–0.93)	0.93 (0.70–0.99)
Cohen´s kappa agreement coefficient	0.24 (0–0.52)	0.24 (0–0.50)	0.63 (0.51–0.75)	0.58 (0.34–0.82)

eral, it was considered less important to clean the "dirty" area of the slaughterhouse thoroughly and it was not included in the sampling protocol. This goes against the hurdle concept, since ignoring contamination of "dirty" areas presumes a sufficient reduction procedure for carcasses before entering the clean side. Overall, the results showed that the efficacy of C&D was better in the processing area than in the slaughter area. This was observed e.g., when comparing the results for the cutting blade for bleeding in the "dirty" slaughter area of the poultry slaughterhouse with those for the cutting blade for thighs in the "clean" processing area. Another example of an inadequately cleaned surface was the lairage floor, presumably because of limited time between the slaughter shifts at the poultry slaughterhouse, caused by a high number of birds slaughtered each day, which left little time for C&D procedures (2–4 h). There was only time for rinsing feces and foaming with detergent before new birds arrived at the lairage.

Visible dirt was observed after C&D on the post-dehairing table in the red meat slaughterhouse. In half of the samples after C&D, this surface had very high TAB values. High TAB count (3.8 log CFU/cm^2) has also been observed on post-dehairing tables in other studies (Rivas et al., 2000). It is a major concern when such a FCS is insufficiently cleaned, since it creates the risk of cross-contamination of meat (Okpo et al., 2015; Samapundo et al., 2019). The red meat slaughterhouse included in this study had problems with high TAB values on pig carcasses, which were believed to be caused by the insufficiently cleaned post-dehairing table.

High TAB values were found for the drains and the conveyor belt for pig organs after C&D in the red meat slaughterhouse. The conveyor belt looked worn and displayed large scratches, which could harbor bacteria. However, these surfaces were NFCS and were not as critical for food safety as FCS. However, if NFCS such as drains still contain a high amount of bacteria after C&D, a resident house microbiota could be created. A particular *L. monocytogenes* strain has been found to persist for many years in a drain in a Norwegian food processing plant (Fagerlund et al., 2016). Remaining resident bacteria could be transferred from NFCS to FCS when rinsing the drains if contaminated aerosols land on nearby FCS such as conveyor belts (Saini et al., 2012).

Both conveyor belts located in the processing area of the poultry slaughterhouse (sampling point 17 was smooth with lumps, sampling point 18 was modular) were successfully cleaned. These surfaces had similar TAB reductions to those reported for clean conveyor belts in another study (Gómez et al., 2012), although it is unclear whether the slaughterhouse examined in that study was for poultry or red meat. The TAB reductions for the conveyor belts in the poultry slaughterhouse in the present study were higher than those observed in a beef processing plant (Wang et al., 2018), which is surprising since that study examined manual scrubbing and drying of surfaces, which should improve the C&D procedure, compared to the present study where in general, manual scrubbing was not used. In a beef processing plant in another study, C&D of a conveyor belt in a cutting room did not lead to a significant reduction in CFU, and large amounts of bacteria were still present even after rigorous C&D (Khamisse et al., 2012). In the present study, the level of cleanliness of the two conveyor belts in the poultry slaughterhouse was deemed acceptable based on the ATP-bioluminescence results, which were similar to those in another study performed in a poultry slaughterhouse (Rodrigues et al., 2018). In the present study, EB could not be detected on conveyor belts after C&D, which is in agreement with the findings by Wang et al. (2018). Possible reasons are that the cleaning staff prioritized cleaning conveyor belts and that these particular FCS mostly had intact surfaces without scratches. It should also be mentioned that, especially in the processing area of the poultry slaughterhouse, the cleaning staff were aware of the time and location of the sampling procedure, which could have influenced the results.

Enterobacterales could be enumerated in more samples before C&D in the poultry slaughterhouse than in the red meat slaughterhouse. This may indicate that the poultry slaughtering process causes a higher level of fecal contamination of surfaces than the slaughter of cattle and swine. Greater amounts of *E. coli*, EB, and TAB on poultry meat compared with pork and beef have been observed in a previous study (Ghafir et al., 2008). This is most likely due to intestinal rupture during slaughter occurring more often in poultry slaughter. Moreover, more water is used when slaughtering poultry compared with slaughtering cattle and pigs, which facilitates the spread of bacteria (Adams & Moss, 1995; Ninios et al., 2014). However, the TAB reductions in the present study were higher in the processing area of the poultry than in the red meat slaughterhouse (2.8 vs. 2.2 log CFU/100 cm^2), indicating a stronger C&D effect. The slaughterhouses used similar C&D products and procedures, except that the poultry slaughterhouse used 10–15°C higher water temperature for the C&D procedure. Other factors that could also have influenced the results included the type of meat, surface, material, and wear and tear.

To enable evaluation of the performance of the dipslide and ATP-bioluminescence methods in comparison with swabbing and plating (TAB), acceptable thresholds had to be selected for each sampling method to decide whether a surface could be considered acceptably clean. Conventional swabbing and plating was chosen as reference, because it is a widely accepted bacteriological method and can be used to swab places difficult to reach (Griffith, 2016). It was not possible to compare ATP-bioluminescence with EB, since the latter is more specific. It is important to emphasize that there are no standardized thresholds for when a surface is sufficiently clean at the European or national level (Sweden). Even the European standard, which should be applied in sampling and analysis (SS-EN ISO 18593:2018), does not mention thresholds or specify how to interpret the results. Thus, each FBO selects thresholds based on trends measured over time and different FBOs use different thresholds. In this study, the same thresholds for evaluating cleanliness were used for both slaughterhouses, and for both FCS and NFCS. However, a FBO may decide to accept a greater amount of bacteria/organic debris on NFCS and use different

thresholds on different surfaces. Based on the results of the present study, lowering the threshold for the dipslide method and increasing the threshold for the ATP-bioluminescence method should possibly be considered for the red meat slaughterhouse. In this study, two assessment outcomes were used (acceptably and non acceptably clean), but some slaughterhouses also use marginal ranges with values in between the acceptable and non acceptable values. It should be noted that the greater sampling area in swabbing and plating could also have impacted the microbial concentration compared with the other methods studied.

The indicators of diagnostic performance used here have previously been used in other studies comparing different methods for monitoring cleaning and disinfection in food premises (Carrascosa et al., 2012; Ching et al., 2021) and in health care settings (Luick et al., 2013). In the present study, the dipslide method showed lower sensitivity (Se = 0.26) and agreement (κ = 0.24) in the red meat slaughterhouse than in the poultry slaughterhouse (Se = 0.81, κ = 0.63). Carrascosa et al. (2012) observed similar agreement between dipslide and TAB (contact plates) (κ = 0.59) in dairies as seen in the poultry slaughterhouse in the present study. They also found that the dipslide method detected fewer unacceptably clean surfaces than ATP-bioluminescence. The lower sensitivity and agreement in the red meat slaughterhouse could be due to the large difference between the slaughter process for cattle/swine and poultry. The system in the poultry slaughterhouse was mainly automatic, where hanging rotating blades cut the meat, while the system in the cattle/swine slaughterhouse was manual, using, i.e., cutting boards, which resulted in more cuts/cracks in which bacteria could hide. The dipslides did not reach those areas, which could be the reason for the high number of false negatives for that method compared with swabbing and plating. This suggests that dipslides may not be scientifically appropriate for drawing conclusions pertaining to the efficacy of C&D and for determining appropriate microbiological hygiene on cutting boards in cattle and swine slaughterhouses. Other limitations with dipslides are that it is difficult to ensure that the entire agar is pressed on the surface, risking lower detachment of bacteria from the surface, and the lack of mechanical pressure compared with swabbing, which may lead to less bacteria being sampled from the surface. Furthermore, the dipslide method is semi-quantitative, because it is difficult to quantify exactly the number of bacterial colonies when large numbers of bacteria are present, due to non dilution of the sample (Griffith, 2005). However, dipslides are relatively cheap, easy to use, and can be incubated by the FBO.

Low specificity was observed for the ATP-bioluminescence method in both slaughterhouses (0.30 and 0.64 for the red meat and poultry slaughterhouse, respectively). A previous study comparing ATP-bioluminescence with TAB after C&D in a health care setting (Luick et al., 2013) observed higher positive predictive value (PPV = 0.90) and lower negative predictive value (NPV = 0.20) than in the present study (PPV 0.54 and NPV 0.89 for the red meat slaughterhouse, and PPV 0.71 and NPV 0.93 for the poultry slaughterhouse). This was not surprising, since ATP-bioluminescence detects not only bacterial cells but also other cells from organic debris such as blood cells, fat cells, etc. This means that even if bacteria were killed during the C&D process, remaining organic residues could still be detected on the surfaces sampled. This was observed for the cutting blade for carcasses, which was partly covered with burned residues after C&D, which had low TAB values (1.5 log CFU/100 cm^2) but high ATP values (3.9 log RLU/100 cm^2). Another issue to be aware of when using ATP-bioluminescence is that, depending on the type of organic debris present on a surface, the results vary greatly (Lane et al., 2020; Møretrø et al., 2019). Therefore, this method should not be used to assess microbial cleanliness, but can determine the efficacy of C&D, indicating whether or not a surface is clean (Griffith, 2016). Additionally, ATP-bioluminescence is a fast method for monitoring cleanliness, since it provides a result within seconds and thereby enables immediate corrective action, so it is very useful for monitoring C&D before slaughter starts in the morning.

The scald water in the poultry slaughterhouse had higher TAB and RLU values than that in the red meat slaughterhouse, both before and after C&D. One explanation could be the lower temperature of scald water in the poultry slaughterhouse. High bacterial loads pose a risk of cross-contamination of carcasses submerged in the scald water. Studies on the level of hygiene indicator bacteria in scald water during the past 15 years are lacking and few samples have been analyzed in previous studies. In two small-scale poultry slaughterhouses in South Africa (Geornaras et al., 1995, 1997), scald water with similar temperature as in the present study (52–54°C) sampled during production (approximately 2 h after start-up) showed approximately 1.0 log CFU/mL higher EB values, but 1.0 log CFU/mL lower TAB values than scald water sampled in the poultry slaughterhouse in the present study. In another previous study of a small-scale poultry slaughterhouse (Whyte et al., 2004), the levels of TAB and EB in scald water before and after slaughter were approximately 1.0 log CFU/mL higher than observed in the present study. Thresholds for an acceptable level of cleanliness of scald water are lacking, so the scald water was excluded from the comparative assessment of the different sampling methods.

In conclusion, the results obtained in this study highlight the main difficulties for FBOs using common hygiene monitoring methods for assessing surface cleanliness after C&D. These include the risk of missing bacteria when using only dipslides and the difficulty of interpreting the ATP-bioluminescence results, as this method does not only measure the microbial load. Since our results indicate that neither ATP-bioluminescence nor dipslides provide accurate estimates of C&D efficacy when used separately, a possibility would be to combine them. Swabbing and plating (TAB) could be used to verify the reliability of the other methods. Additionally, there is difficulty in interpreting the results of monitoring operations, due to the absence of commonly agreed guidelines on when a surface is sufficiently cleaned. It is also concerning that the slaughterhouses included in this study put less effort into monitoring the cleanliness of FCS in slaughter areas, even though these surfaces may constitute the greatest risk to meat cross-contamination, considering direct contact with the product. This may increase the risk of epidemiological spread of bacterial foodborne pathogens to consumers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Swedish Farmers' Foundation for Agricultural Research (grant numbers O-18-20-158 and O-20-20-447) and the Royal Swedish Agricultural Academy (grant number CF2020-0011). The authors gratefully acknowledge the slaughterhouses that participated in the study, Lise-Lotte Fernström for excellent laboratory assistance and Claudia von Brömssen for statistical advice.

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