

## EVALUATION OF THE DISINFECTION EFFECTIVENESS AT MEAT PROCESSING BY THE TWO METHODS

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### ABSTRACT

The aim of this article was the control of disinfection in selected meat processing-plant by two methods – swabbing method and The 3M™ Petrifilm™ plates. Samples were collected within three months (September, October, November 2014). Together 54 samples selected surfaces were collected. Each month six samples were collected using imprint method with Petrifilm plates on total count of microorganisms, 6 samples using imprint method with Petrifilm plates on the number of coliforms and 6 samples using sterile swab method. Samples were collected from the workbench, conveyor belt, cutting blades, meat grinder, wall and knife. Total viable counts (TVC) and coliform bacteria (CB) were determined in samples. The values of TVC in September obtained by swabbing method were higher than allowable limit in sample no. 1 ( $3.70 \times 10^2$  CFU.cm<sup>-2</sup>) and in sample no. 4 ( $3.35 \times 10^1$  KTJ.cm<sup>-2</sup>). The values of TVC obtained by Petrifilm plates were lower than 10 CFU.cm<sup>-2</sup> in all samples. Values of CB obtained by Petrifilm plates were lower than 1 CFU.cm<sup>-2</sup> in all samples, CB obtained by swabbing method was  $1.6 \times 10^1$  CFU.cm<sup>-2</sup> in sample no. 6. Values of TVC obtained in October by Petrifilm plates and also by swabbing method were higher than permissible limit ( $< 10$  CFU.cm<sup>-2</sup>) in sample no. 2 and sample no. 4. Values of CB obtained by Petrifilm plates were lower than 1 CFU.cm<sup>-2</sup> in all samples, CB obtained by swabbing method was  $3.65 \times 10^1$  CFU.cm<sup>-2</sup> in sample no. 3, value of CB obtained by swab method in sample no. 3 does not meet requirements of internal company standards. Values of TVC obtained in November by Petrifilm plates were lower than 10 CFU.cm<sup>-2</sup> in all samples. TVC obtained by swab method were  $1.25 \times 10^1$  CFU.cm<sup>-2</sup> in sample no. 3 and  $3.25 \times 10^1$  CFU.cm<sup>-2</sup> in sample no. 4. Samples were not in accordance with requirements of internal company standards. Values of CB obtained in November by Petrifilm plates and by swabbing method were after disinfection lower than 1 CFU.cm<sup>-2</sup> in all samples.

**Keywords:** disinfection, total viable count, coliform bacteria, Petrifilm plates, swabbing method

### INTRODUCTION

Many food pathogenic and spoilage bacteria are able to attach to food contact surfaces (Fonnesbech Vogel *et al.*, 2001), and remain viable even after cleaning and disinfection (Frank and Koffi, 1990). Such adhered bacteria can detach during production and contaminate food as it passes the surfaces. This can seriously effect the quality and safety of the processed food and pose a potential risk to the consumer, particularly if contamination occurs after a bactericidal step, such as cooking.

In order to ensure the highest possible hygiene standards in meat, poultry and egg factories, it is essential that each one is cleaned and disinfected regularly. In most factories, this is carried out at the end of the day's processing. Production is stopped, gross debris is removed and then the plant is cleaned with detergents, before being rinsed with water and disinfected. Many factories also clean during staff breaks and this is known as 'mid-shift cleaning'. The effectiveness of cleaning can be extremely variable. It will depend on the training and commitment of the personnel, both those on the production lines and others responsible for the cleaning process itself. In a well-run factory, sufficient time is set aside to ensure a thorough and effective clean, and the whole process is properly documented, using appropriate check lists; furthermore, the cleaning operatives are specially trained, thus providing maximum effectiveness. The importance of ensuring that the whole factory is thoroughly cleaned and disinfected cannot be over-emphasised. The aim of this study was to compare two methods of disinfection control at meat processing (Rigarlsford, 2007).

Cleaning and disinfection (C&D) are among the most important hazard control measures in the meat industry, it is thus of prime importance to assess their efficacy. However, this is a difficult task because C&D cannot remove all microbial cells present on open surfaces. Efficacy of C&D can be assessed by

agar contact plating, a convenient and frequently used method that nevertheless underestimates CFU counts on surfaces (Bagge-Ravn *et al.*, 2003).

Swabbing surfaces to detach microbial cells for quantification is more accurate, but does not remove all cells (Oulahal-Lagsir *et al.*, 2000). Efficiency of swabbing depends on the operator, the swabbing material, the microbial species and the history of the attached cells.

In the food industry, European legislation requires that surfaces should be "in a sound condition and must be easy to clean". Microbiological surface control is ensured in the food process environment provided it is hygienically designed and that adequate hygiene procedures are implemented (Verran *et al.*, 2001). However, insufficient cleaning and disinfection of food contact surfaces represent a potential risk of cross-contamination to food (Mattila *et al.*, 1990). Therefore, any lack of hygiene management may lead to a reduction in food shelf-life with adverse economic and public health consequences (Moore and Griffith, 2002a). Several studies have shown that various foodborne pathogens such as *Escherichia coli*, *Staphylococcus aureus* or *Listeria monocytogenes* can survive for hours or even days on hands, sponge/cloths, or utensils (Kusumaningrum *et al.*, 2003; Wilks *et al.*, 2005). Various methods of detection and enumeration of microorganisms from surfaces are currently available. Microbiological methods including hygiene swabbing or agar contact methods are traditionally employed to assess the cleanliness of surfaces. However, despite the simplicity of use, microbiological testing is limited by incubation steps of 24–48 h whereas results may be needed earlier, ideally prior to the release of the food product to market.

The aim of the present study was to compare two methods of disinfection control at meat processing.

## MATERIAL AND METHODS

The aim of this article was to compare the swab method and fingerprint method, which are used to control of disinfection efficacy in food industry. Control of disinfection efficacy was carried out in the meat processing. Fingerprints by Petrifilm plates and swabs were carried out after disinfection from the following surfaces: workbench – sample no. 1, conveyor belt – sample no. 2, cutting blades – sample no. 3, meat grinder – sample no. 4, wall – sample no. 5, knife – sample no. 6. Total viable counts (TVC) and coliform bacteria (CB) were determined after disinfection of selected surfaces. Sodium hypochlorite (4.7 %) was used to disinfection of surfaces.

### Taking of samples

Together 54 samples were taken from selected surfaces after disinfection during three month. There were taken six fingerprints by Petrifilm plates to determination of TVC, six fingerprints by Petrifilm plates to determination of CB and six swabs each month after disinfection.

### Determination of cfu counts by Petrifilm plates

The Petrifilm Aerobic Count plate is a reliable, ready made medium system for enumerating total aerobic bacteria populations. Petrifilm Aerobic Count plates contain Standard Methods nutrients, a cold water soluble gelling agent and a tetrazolium indicator dye which facilitates colony enumeration.

Petrifilm plates contain special media that must be hydrated before application. Hydration is carried out by pipetting 1 ml peptone solution to the middle of the Petrifilm plate. Fingerprints were performed by placing Petrifilm plates to examined surface. Cultivation was carried out in thermostats at temperature 30 °C for 48 hours (determination of TVC) and at 37 °C for 24 hours (determination of CB). All red dots regardless of size or intensity should be counted as colonies. The circular growth area is approximately 20 cm<sup>2</sup>. The results were converted to CFU.cm<sup>-1</sup>.

### Determination of cfu counts by swab method

Sterile swabs were used for the sampling from surfaces and equipments. Swabs were placed in a test tube with 9 ml of physiological solution. Swabs were performed by gentle rubbing in area 20 cm<sup>2</sup> from controlled surface. Swabs with samples were placed in a transport medium. Dilution plating method was used to determine the microorganisms. Inoculation was performed with a sterile pipette, 1 ml of triple repeats (parallel to the three Petri dishes) for each dilution used. Dilutions 10<sup>-3</sup> and 10<sup>-4</sup> were used to determination of total viable counts.

Plate Count Agar was used for determine of Total Viable Counts in samples. Petri dishes were cultivated upside-down in a thermostat at 30 °C for 48-72 hours under aerobic conditions. Dilutions of 10<sup>-1</sup> and 10<sup>-2</sup> were used to determine the number of coliform bacteria. Violet red bile agar was used for determine of Coliform Bacteria in samples. Petri dishes were cultivated upside-down in a thermostat at 37 °C for 24 -48 hours. The results were converted to CFU.cm<sup>-1</sup>.

### Evaluation of CFU counts

Characteristic colonies on the Petri dishes and the Petrifilm plates were counted after cultivation. Results were evaluated according to Regulation of Slovak Republic (SR) no. 281/2003 as follows:

TVC - acceptable range – 0-10 CFU.cm<sup>-2</sup>  
CB - acceptable range - 0-1 CFU.cm<sup>-2</sup>

## RESULTS AND DISCUSSION

Control of disinfection was carried out at meat processing during three month (September 2014, October 2014 and November 2014). Two methods of disinfection control were compared – swab method and method by Petrifilm plates.

Bacterial recovery from surfaces using swabbing techniques may be influenced by several factors such as the ability of the swab to remove the microorganisms from the surface, their effective release from the swab and subsequent recovery using plating or other techniques (Moore and Griffith, 2002a).

### Evaluation of microorganisms in September 2014

The values of TVC obtained by Petrifilm plates were lower than 10 CFU.cm<sup>-2</sup> in all samples. Values of TVC obtained by swab method were 3.70×10<sup>2</sup> CFU.cm<sup>-2</sup> in sample no. 1 (workbench) and 3.35×10<sup>1</sup> CFU.cm<sup>-2</sup> in sample no. 4 (meat grinder). TVC in samples no. 2, 3, 5 and 6 were lower than 10 CFU.cm<sup>-2</sup> (tab. 1). Values of TVC in sample no. 1 and 4 were not in accordance with requirements of Regulation of SR no. 281/2003.

The removal of bacteria from dry surfaces can be improved using a pre-moistened swab as confirmed by Moore and Griffith (2002b). In the present

study, swabs were impregnated with a commercial solution (SRK) or Ringer ¼ strength solution. The SRK solution contained in a swab tube is a non-nutrient phosphate buffered solution purporting to include substances that neutralize and inactivate disinfectants and sanitizing agents. This non-nutrient, phosphate buffered solution enables quantification of microorganisms when transported at ambient temperature.

Bazaco et al. (2007) advised the use of Copan SRK solution for quantifying microbial recoveries as confirmed by Moore and Griffith (2007) for the recovery of *E. coli* and *S. aureus*. Moreover, the use of cotton swabs associated with Ringer ¼ strength solution has been demonstrated to be advantageous (Moore and Griffith, 2002c). Analysis of naturally contaminated surfaces generally involves the use of traditional plate counting or 3M Petrifilm™. The latter is frequently used by the food industry for the enumeration of *L. monocytogenes*, *S. aureus* and the Enterobacteriaceae in foods or from surfaces with bacterial recoveries comparable to traditional plating methods (Fedio et al., 2008; Paulsen et al., 2008 and Vicosa et al., 2010).

**Table 1** Determination of TVC in September 2014

Samples	TVC (CFU.cm <sup>-2</sup> )	
	Petrifilm plate	Swab method
1	< 10	3.70×10 <sup>2</sup>
2	< 10	< 10
3	< 10	< 10
4	< 10	3.35×10 <sup>1</sup>
5	< 10	< 10
6	< 10	< 10

Values of CB obtained by Petrifilm plates were lower than 1 CFU.cm<sup>-2</sup> in all samples, CB obtained by swab method was 1.6×10<sup>1</sup> CFU.cm<sup>-2</sup> in sample no. 6 (knife), all others samples were negative for presence of coliform bacteria (tab. 2). Only sample no. 6 does not meet requirements of Regulation of SR no. 281/2003.

In the study of Martinon et al. (2012) data showed that *L. monocytogenes* was not detected but some non-presumptive colonies were found on 3M Petrifilm™ *Listeria* Count Plate and LSA for 4 samples collected inside a food filling dispenser within the factory. It is reasonable to suggest that the microorganism involved may be *Listeria* species but not *L. monocytogenes*. *S. aureus* was not detected either, despite the presence of black colonies on Baird Parker; however these tested coagulase negative, and originated from a sample collected inside a large empty food container undergoing a cleaning process. However, the presence of Enterobacteriaceae, using plating and Petrifilm™ methods, was confirmed from surface samples. Characteristic colonies of Enterobacteriaceae were observed in samples collected from hoppers, exposed machine surfaces or cylinders of machines. The tests performed on the subcultures showed that the bacteria were oxidase negative and positive for glucose fermentation.

**Table 2** Determination of CB in September 2014

Samples	CB (CFU.cm <sup>-2</sup> )	
	Petrifilm plate	Swab method
1	< 1	< 1
2	< 1	< 1
3	< 1	< 1
4	< 1	< 1
5	< 1	< 1
6	< 1	1.6×10 <sup>1</sup>

The quantification of Enterobacteriaceae was not available for a majority of naturally contaminated samples, as parts of machinery were not flat so a template could not be applied. Some studies have related issues associated with bacterial recoveries from dry surfaces using swabbing techniques (Davidson et al., 1999) Moore and Griffith (2002c) explained that the efficiency of the swabbing technique was lower when sampling dry surfaces compared with wet surfaces as microbial viability may be affected by drying.

### Evaluation of microorganisms in October 2014

Values of TVC obtained in October by Petrifilm plates were 1,6×10<sup>1</sup> CFU.cm<sup>-2</sup> in sample no. 2 (conveyor belt) and 1,2×10<sup>1</sup> CFU.cm<sup>-2</sup> in sample no. 4 (meat grinder). Values were higher than permissible limit (< 10 CFU.cm<sup>-2</sup>). TVC in samples no. 1, 3, 5 and 6 were lower than 10 CFU.cm<sup>-2</sup>. TVC obtained by swab method were 4,35×10<sup>1</sup> CFU.cm<sup>-2</sup> in sample no. 2 and 2,48×10<sup>2</sup> CFU.cm<sup>-2</sup> in sample no. 4, TVC in samples no. 1, 3, 5 and 6 were lower than 10 CFU.cm<sup>-2</sup> (tab. 3). These results are similar to results obtained by Petrifilm plates.

**Table 3** Determination of TVC in October 2014

Samples	TVC (CFU.cm <sup>-2</sup> )	
	Petrifilm plate	Swab method
1	< 10	< 10
2	1.6×10 <sup>1</sup>	4.35×10 <sup>1</sup>
3	< 10	< 10
4	1.2×10 <sup>1</sup>	2.48×10 <sup>2</sup>
5	< 10	< 10
6	< 10	< 10

Values of CB obtained by Petrifilm plates were lower than 1 CFU.cm<sup>-2</sup> in all samples, CB obtained by swab method was 3.65×10<sup>1</sup> CFU.cm<sup>-2</sup> in sample no. 3 (cutting blades), coliform bacteria were lower than 1 in other samples (tab. 4). Value of CB obtained by swab method in sample no. 3 does not meet requirements of Regulation of SR no. 281/2003.

Jessen and Lammert (2003) measured the aerobic counts of cleaned and disinfected surfaces of processing lines for sliced cooked ham products and found values as high as 10<sup>4</sup> CFU.cm<sup>-2</sup>. Similarly, Bizzaro et al. (1990) found more than 10<sup>4</sup> CFU.cm<sup>-2</sup> on cleaned and disinfected polyurethane conveyor belts for delicatessen products.

**Table 4** Determination of CB in October 2014

Samples	CB (CFU.cm <sup>-2</sup> )	
	Petrifilm plate	Swab method
1	< 1	< 1
2	< 1	< 1
3	< 1	3.65×10 <sup>1</sup>
4	< 1	< 1
5	< 1	< 1
6	< 1	< 1

Marouani-Gadri et al. (2009) investigated the effects on *Escherichia coli* O157:H7 biofilm formation of bacteria isolated from meat site surfaces following cleaning and disinfection. Samples were obtained by swabbing the surfaces of equipment or floors over areas ranging from 315 to 3200 cm<sup>2</sup> in a slaughter hall, a meat cutting room and a meat boning room of a meat-processing plant. The number of bacteria recovered from these surfaces ranged from < 1 to > 10<sup>5</sup> CFU.cm<sup>-2</sup>. In the slaughter hall, stainless steel was in one case one of the most contaminated materials and in other cases one of the less contaminated. The same observation was made for conveyor belts made of polyvinyl chloride in the boning room. Dominant genera in the meat plant were *Staphylococcus* and *Bacillus* which were both 34 % of the isolates from the slaughter hall and 14 and 4 % respectively of the isolates from the cutting room. Randomly selected isolates of each of the genera recovered from the slaughter hall were cultured with *E. coli* O157:H7 in meat exudate at 15 °C to form dual-organism biofilms on polyurethane. In all cases but one, the isolates increased the numbers of attached *E. coli* O157:H7. The effects ranged from 0.37 to 1.11 for EDL 933 strain and from 0.19 to 1.38 log (CFU.cm<sup>-2</sup>) for Sakař strain. This is the first time that a resident microbiota of a meat-processing plant has been shown to have a favourable effect on *E. coli* O157:H7 colonization of a solid surface, which is of great interest from a food safety standpoint.

#### Evaluation of microorganisms in November 2014

Values of TVC obtained by Petrifilm plates were lower than 10 CFU.cm<sup>-2</sup> in all samples. TVC obtained by swab method were 1.25×10<sup>1</sup> CFU.cm<sup>-2</sup> in sample no. 3 (cutting blades) and 3.25×10<sup>1</sup> CFU.cm<sup>-2</sup> in sample no. 4 (meat grinder) (tab. 5). Samples were not in accordance with requirements of Regulation of SR no. 281/2003.

The large number of coagulase negative *Staphylococcus* isolates obtained is indicative of their particular ability to colonize materials and/or resist cleaning and disinfection operations. They have been shown to predominate on the defeathering machines of a poultry processing site (Mead and Scott, 1994) and have also been isolated from a variety of food processing environments after cleaning and disinfection (Holah et al., 2002; Mettler and Carpentier, 1998 and Bage-Ravn et al., 2003).

**Table 5** Determination of TVC in November 2014

Samples	TVC (CFU.cm <sup>-2</sup> )	
	Petrifilm plate	Swab method
1	< 10	< 10
2	< 10	< 10
3	< 10	1.25×10 <sup>1</sup>
4	< 10	3.25×10 <sup>1</sup>
5	< 10	< 10
6	< 10	< 10

Values of CB obtained in November by Petrifilm plates and by swabbing method were after disinfection lower than 1 CFU.cm<sup>-2</sup> in all samples (ta. 6).

Aycicek et al. (2006) were used Adenosine triphosphate (ATP) bioluminescence and traditional microbiological swabbing culture methods for detection of surface hygiene on worktops, cutting boards and equipments at a hospital kitchen. In their study, 280 samples from 14 different surfaces in the hospital kitchen were examined such as steel, polyethylene plastic, wooden and marble workbenches (vegetable, meat, bakery preparing), meat grinder, mash machine, polyethylene plastic meat and vegetable chopping workbenches, meat and vegetable knives, gastronomie basin, tap head, oven handle. Of the results, 2.5% that were evaluated "clean" by ATP measurement were confirmed clean by the microbiological technique; on the other hand, 37.1% of the results that were evaluated "clean" by the microbiological technique were determined "dirty" by the ATP measuring technique. 97.5% of the results that were evaluated "clean" by ATP measurement technique was determined "clean" by microbiological technique, on the other hand 62.9% of the results that were evaluated "clean" by the microbiological technique were determined "clean" by the ATP measuring technique.

**Table 6** Determination of CB in November 2014

Samples	CB (CFU.cm <sup>-2</sup> )	
	Petrifilm plate	Swab method
1	< 1	< 1
2	< 1	< 1
3	< 1	< 1
4	< 1	< 1
5	< 1	< 1
6	< 1	< 1

Balzaretti and Marzano (2013) evaluated the hygienic standards of 44 foodservice facilities by monitoring the food contact surfaces (n = 302), and food handlers (n = 287). The hygienic standard of surfaces was sufficiently high. Only 7.9 % of surfaces did not conform with advisory standards in terms of total coliforms, and 2.6 % were found to be contaminated with *Enterococcus* spp. at ≥1.0 log CFU.cm<sup>-2</sup>. The hygienic standard of washed and disinfected hands of food workers was not adequately high: the total bacterial count and coagulase positive *Staphylococci* exceeded the satisfactory limit in 8.4 % and 3.5 % of cases, respectively.

#### CONCLUSION

Total viable count and number of coliform bacteria were determined from selected surfaces at meat processing after disinfection during three month. Two methods for monitoring the efficiency of disinfection were used – swabbing method and 3M<sup>TM</sup> Petrifilm<sup>TM</sup> plates. Our results show, that swabbing method is more sensitive in compare with 3M<sup>TM</sup> Petrifilm<sup>TM</sup> plates.

A major goal for the food processing industry is to provide safe, wholesome and acceptable food to the consumer. Control of microorganisms is essential to meeting this goal. This control is partly exerted through processing and preservation techniques that eliminate microorganisms or prevent their growth.

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