Comparison of different swab techniques for the quantitative analysis of mastitis relevant pathogens on liner surfaces

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Abstract

During machine milking, udder-pathogenic microorganisms can be transmitted. Unclean teats or milking of cows infected with mastitis pathogens can lead to contamination of the liner. To understand pathogen transmission through the liner, it is necessary to identify how many microorganisms adhere to the liner. Therefore, the microorganism density on the inner surface must be determined quantitatively. In this study, a multifactorial laboratory test was used to identify which is the best sampling technique on the liner surface and subsequent diagnostic procedure for quantitative analysis.

Liners were contaminated with several mastitis pathogens using a standardized procedure and four different sampling techniques were applied. Three of these techniques were wet-dry swabs (WDS); the first was performed with high contact pressure, the second with low contact pressure, and the third used high contact pressure with cosmetic swabs instead of swabs from laboratory supplies to reduce costs. These WDS techniques were compared with a dry swab (DS). Contamination was detectable in the used pathogens with all techniques. The use of a WDS is shown to have advantages over the DS, as the contact pressure applied to the swabs and the moisture of the swabs itself play an important role in recovery of pathogens. Thus, with the WDS with high contact pressure, a pathogen recovery of 2.503 $\log_{10}((cfu+1)/cm^2)$ was achieved, while with the DS, only a value of 1.342 $\log_{10}((cfu+1)/cm^2)$ was obtained. Our findings demonstrate that the different sampling techniques differ in their results and that there is a need for standardized sampling of the liner surface to compare the results of the microorganism density quantitatively. The WDS technique in accordance with DIN 10113-1: 1997-07 for determining surface microbial counts in the food industry proves to be a more suitable method than the DS technique due to a higher recovery of microorganisms and uniform results of the determined microbial densities.

Keywords: wet-dry swab technique, mechanical milk removal, mastitis prevention, sampling, bacterial density

Introduction

Udder health of dairy cows is of high economic importance in agriculture. Udder diseases result in decreased milk yield, earlier and increased dairy cow culling, and high veterinary treatment costs for farmers [1; 2; 3]. To reduce treatment costs and animal losses, it is useful to control the spread of mastitis-related pathogens through preventive measures [3; 4]. Since mastitis is a classic factor disease [4; 5], it is important to adopt a broad approach to infection prevention and to take appropriate measures regarding husbandry, hygiene, and milking techniques [6; 7].

In the case of udder-pathogenic microorganisms, a distinction is made according to the classical scheme between environmental and contagious pathogens [4]. Environmental pathogens, such as *Escherichia* (*E.*) *coli*, *Streptococcus* (*Sc.*) *uberis*, and *Sc. dysgalactiae* are mainly transmitted between milkings in the barn and have their reservoir in contaminated bedding material, for example [8]. In contrast, the group of contagious pathogens includes *Sc. agalactiae*, *Staphylococcus (S.) aureus*, and Mycoplasma. Since the reservoir of these pathogens is the mammary gland itself, transmission of these contagious microorganisms occurs mainly during milk removal from udder to udder, through the milking equipment, through milkers' hands and used udder cloths [4; 9]. However, studies have shown that the differentiation between environmental and contagious pathogens remains unclear and that even a classical environment-associated pathogen such as *Sc. uberis* can behave contagiously [10; 11].

During machine milking, the liner, in addition to the milker's hands, acts as an interface between the milking equipment and the cow, so that pathogens are transferred from cow to cow to a considerable extent via the liner [12; 13]. The transfer of classical contagious pathogens to the liner surfaces is mainly favored by pressure differences and air infiltrations occurring during the milking process [14]. Thus, vacuum fluctuations or insufficiently large milk-diverting lines of the system lead to respray effects of the contaminated milk [15; 16]. However, since environmental pathogens also reach the teat skin through contamination, they also come into contact with the inner surface of the liner [17], so that transmission of environmental pathogens during milking would be conceivable. It was already demonstrated that after milking *Sc. uberis*-infected animals, the liner was contaminated accordingly [11] even after another healthy cow had already been milked. Therefore, optimal teat condition, thorough pre-cleaning of the teats, a well-fitting milking cluster, and the hygienic and technically proper

condition of the milking equipment are essential for udder health [18]. This includes, among other things, regular maintenance, servicing and intermediate disinfection of the cluster [19], since mastitis pathogens can adhere to the surface of the liner after infected cows have been milked. According to a previous study, *S. aureus*, for example, can thus be transmitted to the subsequently milked 6-8 cows [20].

Liners are exposed to daily influences such as milk constituents as well as acids and alkalis from cleaning during their lifetime. While silicone liners show good resistance to butterfat, detergents, and disinfectants [21], liners made out of Nitrile Butadiene Rubber (NBR) suffer from the environmental influences. This increases the risk of pathogen transmission when liners overage, as the material becomes increasingly inflexible, especially due to fat deposits [22; 23]. Cracks develop due to the high mechanical stress during the milking process in which microorganisms settle, which are not adequately eliminated during the intermediate disinfection of the milking equipment [24]. These cracks were already demonstrated with electron microscopy by Noorlander & Heckmann [25] on unused teat liners made of rubber materials, whereas liners made of silicone were free of cracks, depressions, ridges, and breaks. After thousands of milkings within their study, the cracks enlarged into caverns in which electron microscopic detection of microorganisms was possible.

Automatic milking systems (AMS) should be viewed particularly critically regarding the potential transmission of pathogens through the liner surfaces. It is important to consider that the automatization of the milking process implements risk factors regarding the transmission of mastitis pathogens. Although it may be an advantage that the contamination of cluster and teat skin by humans is eliminated because the teat preparation is not performed by a human milker anymore, it is disadvantageous that the number of cows milked per cluster increases as well as the milking time. In addition to this, a clear health based milking sequence is, due to the system, not applicable [4; 26; 27; 28].

Due to the large role of liners in pathogen transfer of both contagious and environmental pathogens, quantitative analysis of microbial density on the liner surface should be performed. One way to detect microorganisms on surfaces that are difficult to access, is to use swab methods. These were utilized for hygienic evaluation of milking systems as early as 1941 [29]. They are used to analyze high bacterial counts in milk production or for microbiological control of cleaning and disinfection measures of the liner [24].

However, lack of standardization due to different swab methods as well as swabs and differences in application and laboratory evaluation make the sample techniques insufficient for quantitative analysis and comparison of microbial density on the liner surface in practice [30]. The applied contact pressure, the performing speed of swabbing, the number of swabs, the moisture level of swab and surface and especially the size of the sampled surface are variables to be considered when taking samples in order to obtain a replicable result [24].

Since the liner is the part of the milking equipment that actually touches the cow's teat, it is necessary to be able to determine how many microorganisms adhere to the liner and can thus lead to infection. In practice, different sampling techniques on the liner surface are used, and effects due to different samplers and different sample preparation in the laboratory are neglected. As a result of the different methods, consistent results cannot be obtained and comparison of the microorganism density on the surface is difficult. Therefore, the aim of the study was to describe a standardized method for the practical application of swab samples on the milking cluster for quantitative analysis of the microorganism density on the liner surface. For this reason, the trial tested the performance of different swab methods in terms of their standardizability, replicability, range of variation, and recovery of microorganisms based on a laboratory trial. The results serve as reference values for the suitability of the methods to make reliable statements about the microbial density on the liner surface.

Materials and methods

Contamination of the liners: Samples analyzed in the study were obtained in the laboratory (Hannover University of Applied Sciences and Arts, Hannover, Germany) from NBR (nitrile butadiene rubber) liners (WS029U, Milkrite, Aulendorf, Germany) that had already been used for 2000 milkings on one farm over a seven-month period. These were autoclaved at 121 °C for 20 minutes before being contaminated with four different pathogen isolates of standardized microbial density. Raw milk, previously thermized in a water bath at 60 °C for 50 minutes, was used to contaminate the liner surfaces after inoculation with one pathogen at a time. For inoculation of the liners, the liners were immersed up to their heads to a depth of 9 cm in the milk contaminated with one pathogen species for five seconds before being placed upright on the head on paper to dry. After a 60-second drying period, swab samples were collected using a variety of techniques as stated below.

Pathogens and contamination of the milk: *E. coli* (DSMZ 1300), *S. aureus* (ATCC 700407), *Sc. uberis* (ATCC 12600), and *Sc. agalactiae* (wild isolate, Hannover University of Applied Sciences and Arts, St. No. 1188109) were the four pathogens used in the study. Pathogen isolates were stored at -80 °C with the addition of glycerol until assayed. Precultivation of each pathogen from the stock was performed for 24 h at 37 °C in brain-heart broth. For the main culture, 10 µL of the preculture was again incubated in brain-heart broth at 37 °C for 24 h. A total of 399.6 mL of milk heat treated for 50 min at 60 °C and cooled down to room temperature was contaminated with 0.4 mL of one main culture and thoroughly mixed with a magnetic stirrer to approximate a microbial density of 10^5 colony-forming units per milliliter (cfu/mL) of milk. The actual pathogen content of the contaminated milk was determined culturally using surface methods on a blood agar with aesculin (Fisher Scientific GmbH, Schwerte, Germany).

Sampling techniques: Swab samples were collected using either SWAB A, a sterile individually wrapped swab with a cotton tip for laboratory use (part number 09.119.9100, Paul Boettger GmbH & Co. KG, Bodenmais, Germany) or SWAB B, a cotton swab with a bamboo stick and a tip made of Bisphenol A (BPA) free cotton for cosmetic use (Outdoor Freakz GmbH, Zossen, Germany), which were previously heat sterilized at 140 °C for 2 hours. The swab solution used was 2 mL of one-quarter strength sterile Ringer's solution (Merck KGaA, Darmstadt, Germany). The sampling techniques used were two WDS procedures in accordance with DIN 10113-1: 1997-07, one of the variants was with high contact pressure, one WDS with low contact pressure, and one DS. The WDS samples with high contact pressure were performed using either SWAB A or SWAB B. For the WDS procedure, the cotton head of the first swab was moistened with sterile Ringer's solution by immersing it once into the swab solution for five seconds. Excess moisture was squeezed out at the edge of the test tube. The second swab was used dry, whereas a single dry swab was used for the DS. In the procedure with high contact pressure, a sufficient amount of pressure was applied for the swab to bend, whereas in the procedure with low contact pressure, the wooden handle did not bend. The DS was also carried out with high contact pressure. All techniques had in common, that the cotton tip of the swab was passed once along the inside of the liner at a depth of 5 cm. The swab itself was turned while doing this. In order

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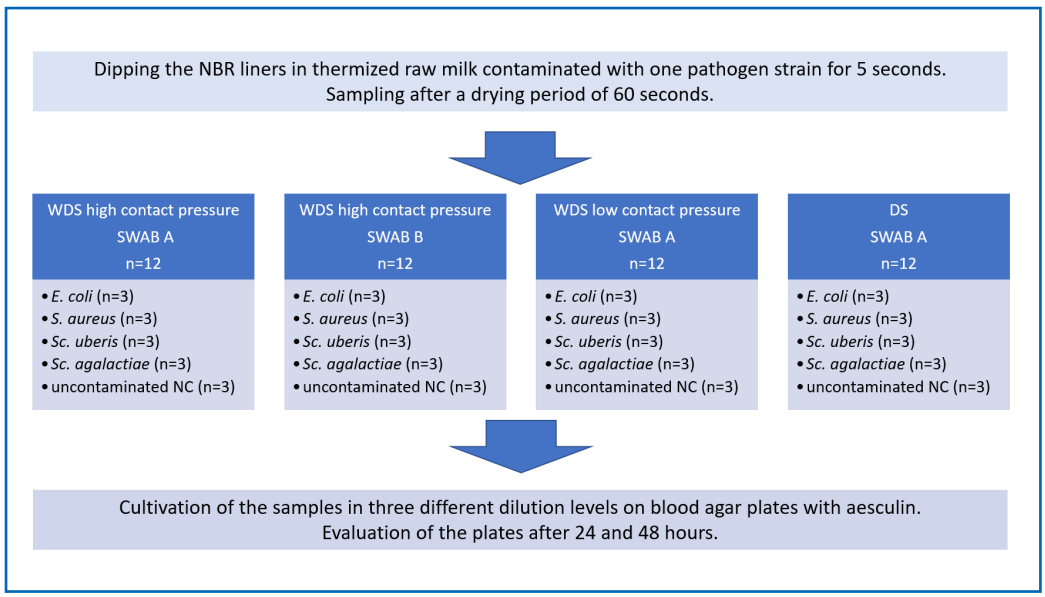

Figure 1: Diagram of the experimental set-up. WDS: wet-dry-swab, DS: dry-swab, SWAB A: for laboratory use, SWAB B: for cosmetic use, NC: negative control

to accurately define the swabbing area, the circumference of the inside of the liner was swabbed once. Each of the variations was performed in triplicate. In addition, all sampling techniques were supplemented with a negative control in which the liners were immersed in uncontaminated, thermized raw milk. The contamination density of the milk was characterized by a positive control.

Samples: 12 samples were collected using each of the four different swab sampling techniques, with three replicates made using each of the different pathogens. Thus, a total of 48 samples were taken from contaminated liners. In addition, the negative control described above was performed (Figure 1).

Microbiology: For preparation, samples of 2 mL Ringer's solution and the swabs used were shaken for 60 seconds with a vortex mixer to transfer the germs sticking to the swab head into the liquid medium to obtain an initial dilution before a decimal dilution series was made in accordance with §64 LFGB (German Food and Feed Code) method: L 00.00-54. For homogenization, the samples were mixed again before the sample fluids were spread in several dilution steps (10^{-1}, 10^{-2} and 10^{-3}) in duplicate on blood agar with added aesculin (Fisher Scientific GmbH, Schwerte, Germany) for microbial count, using the spatula method. After incubation at 37 °C for 24 and 48 hours, the plates were evaluated using the macro-colony counting method, considering all plates that showed growth of between 10 and 300 colonies. The weighted arithmetic mean in cfu/mL of all evaluable dilution levels of a sample determined in accordance with §64 LFGB, method L 01.00-57 was converted into cfu per square centimeter (cfu/cm²) in adaptation to the swabs used, the applied contact pressure and the thus varying swabbed surface. For the calculation, the 7.85 cm circumference of the inside of the liner at a depth of 5 cm was taken and multiplied by the contact area of the swab tips.

Statistics: The obtained data were collected in Microsoft Excel and analyzed using the SPSS 28.0 program, SPSS Inc. (Chicago, IL, USA). The outcome variable 'cfu/cm² of a pathogen' was transformed to approximate a normal distribution and tested with the Kolmogorov-Smirnov test. Associated factors with the outcome variable were identified with an analysis of variance and post hoc analysis using the Fisher least significant difference (LSD) test to reveal significant differences between group means. Statistical significance was defined as $p < 0.05$.

Evaluation: All process steps that could be standardized were done so as described above. This concerned the preparation of the contaminated milk, the immersion time and depth of the liners, the drying time of the liners before sampling, the depth of sampling, the handling of the swab, the shaking of the swabs in their medium as well as the cultivation and evaluation of the samples. These steps were performed to ensure that consistent results are obtained in subsequent studies of the same or similar questions when using the standardized process. In order to compare the individual swab procedures, the recovery of the bacteria was taken into account, i.e., the ability of the swab to collect the bacteria from the surface of the liner under applied contact pressure and used moisture. As a further measure for comparing the swab methods and also for standardization and repeatability of the method, the range (V_{max} - V_{min}) of the results was used. The smaller this range, the more uniform the results obtained. With a small range of variation it can be assumed that similar results will be obtained if the study is repeated.

Results

In the experiment, including the negative control, a total of 60 samples were run using the four different swabbing methods and subjected to microbiological analysis. The cfu were counted after 24 and 48 h of incubation. The total bacterial count in the contaminated milk differed slightly between the bacterial species. Thus, a bacterial density in the milk of $1x10^6$ cfu/mL was achieved for *E. coli*, $4x10^5$ cfu/mL for *S. aureus*, $3x10^5$ cfu/mL for *Sc. uberis*, and $5x10^5$ cfu/mL for *Sc. agalactiae*. In the evaluation of the negative controls, no colony growth was detectable, so that contamination of the swabs or liners could be excluded. To exclude contamination by the hands of the sampler, the handles of the swabs were broken off when they were transferred to the sample container. As the cosmetic swabs also had bamboo handles, this was also possible with SWAB B.

Due to the use of different swabs and thus the different sizes of the cotton tips as well as the different contact pressures applied, the sampled areas varied in size from 7.06 cm² (WDS with high contact pressure and DS using SWAB A), 3.90 cm² (WDS with low contact pressure using SWAB A), and 6.28 cm² (WDS with high contact pressure using SWAB B). To compare the different sampling techniques, the results were converted from cfu/mL to cfu/cm² using the size of the different sampled areas. The collected data did not show a normal distribution and were therefore normalized by adding 1 and applying the $\log_{10}$ transformation. According to this, the conditions for the acceptance of

Table 1: Mean bacterial counts for the different sampling techniques and different pathogens in $\log_{10}((cfu+1)/cm^2)$.

Sampling technique	N	Mean [$\log_{10}$ ((cfu+1)/cm^2)]	95 % CI Lower Bound	95 % CI Upper Bound
WDS high contact pressure, SWAB A	12	2.503	2.216	2.791
WDS low contact pressure, SWAB A	12	2.189	1.901	2.476
WDS high contact pressure, SWAB B	12	2.497	2.209	2.784
DS, SWAB A	12	1.342	1.054	1.629
Pathogen				
E. coli	12	2.686	2.398	2.973
S. aureus	12	1.901	1.614	2.189
Sc. uberis	12	1.630	1.343	1.918
Sc. agalactiae	12	2.314	2.026	2.601
All samples				
Contaminated liners	48	2.133	1.893	2.373
Negative control	12	0	0	0

WDS: wet-dry-swab, DS: dry-swab, SWAB A: for laboratory use, SWAB B: for cosmetic use

a normal distribution hypothesis were given.

In the 48 samples taken from contaminated milk, the mean bacterial count per cm^2 after contamination was 2.133 $\log_{10}((cfu+1)/cm^2)$. The highest detection of microorganisms was achieved with the WDS with high contact pressure using SWAB A (2.503 $\log_{10}((cfu+1)/cm^2)$, followed by the application of the WDS when using SWAB B (2.497 $\log_{10}((cfu+1)/cm^2)$). The lowest detection thereof was obtained using the DS and SWAB A (1.342 $\log_{10}((cfu+1)/cm^2)$) (Table 1). With a value of <0.001, all p-values of the comparison between DS and the other swab methods were below the significance level of 0.05. In contrast, the mean difference between the WDS with high contact pressure using SWAB A and the WDS with SWAB B differed by only 0.007 $\log_{10}((cfu+1)/cm^2)$ (Table 2).

When the mean values of the different pathogens were considered, the highest pathogen recovery occurred in *E. coli* (2.686 $\log_{10}((cfu+1)/cm^2)$) and the lowest in *Sc. uberis* (1.631 $\log_{10}((cfu+1)/cm^2)$) (Table 1). The values for *Sc. agalactiae* and *S. aureus* were 2.314 $\log_{10}((cfu+1)/cm^2)$ and 1.901 $\log_{10}((cfu+1)/cm^2)$, respectively. Thus, the determined surface bacterial counts differed from each other also due to the different contamination densities in the milk.

Table 3 shows how the results of each sampling technique turned out for the different pathogens. The WDS with high contact pressure using SWAB A achieved the highest results in recovery except for *Sc. agalactiae*. For *Sc. agalactiae* the highest detection (2.829 $\log_{10}((cfu+1)/cm^2)$) was achieved with SWAB B. The DS method consistently proved to be the method with the lowest bacterial recovery. In particular, for *Sc. uberis*, there was no evaluable colony growth. The significant differences between application of DS and the other three swab methods in *Sc. uberis* are shown in Table 4. In addition, a significant difference was also found between the application of WDS with high contact pressure using SWAB A and DS in *S. aureus*.

Discussion

When performed in a standardized manner, the various swab methods allow for defined sampling of the liner surface which is suitable for quantitative analysis of pathogen density on the liner surface. To standardize the procedure, all samples were taken at a depth of 5 cm, analogous to the teat length of the cow [31]. The sampling area was more accurately defined by swabbing the circumference of the inside of the liner chamber once with the swab tip.

In order to quantitatively compare the microbial density results, the surface microbial count must be considered [32]. Due to the use of different swabs and thus the different sizes of the cotton tips as well as the different contact pressures applied, the sampled areas varied in size from 7.06 cm^2 (WDS with high contact pressure and DS), 3.90 cm^2 (WDS with low contact pressure) and 6.28 cm^2 (WDS with high contact pressure with SWAB B). For further standardization, all individual steps of the experiment were performed identically for each swab sample as described above, so that no variations in the recovered bacterial count could result from extended or shortened dipping and drying of the liners or preparation in the laboratory. The cosmetic swabs were

Table 2: Mean difference in bacterial counts in relation to the sampling technique in $\log_{10}((cfu+1)/cm^2)$. The mean difference is significant at 0.05 level.

Sampling technique (St1)	Sampling technique (St2)	Mean difference (St1 – St2) [$\log_{10}((cfu+1)/cm^2)$]	p	95 % CI Lower Bound	95 % CI Upper Bound
WDS high contact pressure, SWAB A	WDS low contact pressure, SWAB A	0.315	0.125	-0.092	0.721
	WDS high contact pressure, SWAB B	0.007	0.974	-0.400	0.413
	DS, SWAB A	1.162	<0.001	0.755	1.568
WDS low contact pressure, SWAB A	WDS high contact pressure, SWAB A	-0.315	0.125	-0.721	0.092
	WDS high contact pressure, SWAB B	-0.308	0.132	-0.715	0.098
	DS, SWAB A	0.847	<0.001	0.440	1.253
WDS high contact pressure, SWAB B	WDS high contact pressure, SWAB A	-0.007	0.974	-0.413	0.400
	WDS low contact pressure, SWAB A	0.308	0.132	-0.098	0.715
	DS, SWAB A	1.155	<0.001	0.748	1.561
DS, SWAB A	WDS high contact pressure, SWAB A	-1.162	<0.001	-1.568	-0.755
	WDS low contact pressure, SWAB A	-0.847	<0.001	-1.253	-0.440
	WDS high contact pressure, SWAB B	-1.155	<0.001	-1.561	-0.748

WDS: wet-dry-swab, DS: dry-swab, SWAB A: for laboratory use, SWAB B: for cosmetic use

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Table 3: Mean bacterial counts for the different pathogens using the different sampling techniques in $\log_{10}((cfu+1)/cm^2)$.

Pathogen	Sampling technique	N	Mean $[\log_{10}((cfu+1)/cm^2)]$	95 % CI Lower Bound	95 % CI Upper Bound
E. coli	WDS high contact pressure, SWAB A	3	2.971	2.396	3.546
	WDS low contact pressure, SWAB A	3	2.823	2.248	3.398
	WDS high contact pressure, SWAB B	3	2.905	2.330	3.480
	DS, SWAB A	3	2.044	1.469	2.619
S. aureus	WDS high contact pressure, SWAB A	3	2.654	2.079	3.229
	WDS low contact pressure, SWAB A	3	1.599	1.024	2.174
	WDS high contact pressure, SWAB B	3	2.069	1.494	2.644
	DS, SWAB A	3	1.283	0.708	1.858
Sc. uberis	WDS high contact pressure, SWAB A	3	2.259	1.694	2.834
	WDS low contact pressure, SWAB A	3	2.078	1.503	2.653
	WDS high contact pressure, SWAB B	3	2.185	1.610	2.760
	DS, SWAB A	3	0.000	-0.575	0.575
Sc. agalactiae	WDS high contact pressure, SWAB A	3	2.130	1.555	2.705
	WDS low contact pressure, SWAB A	3	2.255	1.680	2.830
	WDS high contact pressure, SWAB B	3	2.829	2.254	3.404
	DS, SWAB A	3	2.041	1.466	2.616

WDS: wet-dry-swab; DS: dry-swab; SWAB A: for laboratory use, SWAB B: for cosmetic use

applied in the study to check whether it is possible to use them as a cheap alternative on the milking cluster in practice, since adequate sampling is often not carried out for cost reasons. The bamboo handle also allowed the cosmetic swabs used to be broken off below the handle before being transferred to the swab medium, so that contamination by the sampler's hand could be ruled out in this way. As preliminary tests conducted for the study showed, prior sterilization of the cosmetic swabs was mandatory to avoid microbial contamination and thus falsification of the sample results due to their non-sterile manufacturing and packaging.

All pathogens used were detected with the sampling techniques employed in the study. The highest recovery rate of the pathogens was achieved for *E. coli*, the other pathogens could be detected with the different swab methods at a lower percentage. When comparing the different swab methods with each other, the highest recovery rate was achieved with the WDS with high contact pressure with SWAB A. When using SWAB B, comparable results were achieved, with a recovery rate of only 0.24 % less than with SWAB A. That contact pressure and moisture of the swab play a role in pathogen recovery is shown by the results of the WDS with low contact pressure with SWAB A, with which on average 12.54 % fewer microorganisms were recovered than with high contact pressure, as well as the results of the DS, where a 46.38 % lower recovery rate was achieved in comparison to using the WDS with high contact pressure with swabs for laboratory use.

Thus, it can be concluded, as already described by Pfannenschmidt (2003) and Zimmermann (2003) [24; 17], that the amount of contact pressure placed on the liner is a decisive factor when performing the swab sampling, since both a lower recovery of bacteria and a higher

Table 4: Mean difference in bacterial counts of the used pathogens when using the DS compared to other sampling techniques in $\log_{10}((cfu+1)/cm^2)$. The mean difference is significant at 0.05 level

Pathogen	Sampling technique DS	Sampling technique (St)	Mean difference (DS-St) $[\log_{10}(cfu+1)/cm^2)]$	p	95 % CI Lower Bound	95 % CI Upper Bound
E. coli	DS, SWAB A	WDS high contact pressure, SWAB A	-0.927	0.027	-1.740	-0.113
		WDS low contact pressure, SWAB A	-0.779	0.060	-1.592	-0.034
		WDS high contact pressure, SWAB B	-0.861	0.039	-1.674	-0.048
S. aureus	DS, SWAB A	WDS high contact pressure, SWAB A	-1.372	0.002	-2.185	-0.558
		WDS low contact pressure, SWAB A	-0.317	0.434	-1.130	0.497
		WDS high contact pressure, SWAB B	-0.786	0.058	-1.599	0.027
Sc. uberis	DS, SWAB A	WDS high contact pressure, SWAB A	-2.259	<0.001	-3.072	-1.446
		WDS low contact pressure, SWAB A	-2.078	<0.001	-2.891	-1.264
		WDS high contact pressure, SWAB B	-2.185	<0.001	-2.998	-1.371
Sc. agalactiae	DS, SWAB A	WDS high contact pressure, SWAB A	-0.089	0.852	-0.902	0.724
		WDS low contact pressure, SWAB A	-0.214	0.596	-1.027	0.600
		WDS high contact pressure, SWAB B	-0.788	0.057	-1.601	0.025

WDS: wet-dry-swab; DS: dry-swab; SWAB A: for laboratory use, SWAB B: for cosmetic use

variation were achieved when using the WDS with low contact pressure than when using it with high contact pressure. The use of wet swabs also represents an important factor for evaluation of the results, as according to the studies mentioned above the lowest pathogen yield as well as the highest variation in results occurred with the DS method. Inadequate microorganism detection with the DS could lead to a false negative result if the liners are only contaminated to a low degree. The lower recovery and higher variance with DS could be due to sampling with only one swab. Although the same area is swabbed during sampling as with the WDS, the swabbing in WDS is performed twice unlike in DS. Due to the disadvantages of the DS, sampling did not adequately detect *Sc. uberis* contamination of the liner surface. However, recent studies have shown that *Sc. uberis* transmission is not only classically environmental, but also contagious. Since it is one of the most common causes of mastitis [1; 33], detection on the liner is therefore quite relevant in practice.

Despite the advantages that a DS method offers in comparison with WDS methods in terms of practicality, time, and cost savings, both the lower pathogen recoveries and the high variation in results argue against this method.

As the best results were obtained with the WDS with high contact pressure, it should be considered as the method of choice for quantitative analysis of bacterial density on liner surfaces. The use of different sterile swabs for laboratory use as well as commercially available sterilized cosmetic cotton swabs is possible under the conditions described above. For a quantitative analysis, a conversion of the results to the sampled area is necessary, determined on the basis of the respective swab used and the circumference of the liner chamber. To see if the WDS method with high contact pressure is also suitable for quantitative analysis of surface microbial counts on other liner types, the study would need to be repeated with liners made out of other materials.

Conclusion

The study was conducted to determine a suitable swab method for quantitative analysis of surface bacterial counts on the inside of teat liners made of NBR. In order to provide quantitative evidence and make the results comparable, sampling was standardized and results were converted from cfu/mL to cfu/cm^2. Under these standardized conditions, the wet-dry swabbing technique in accordance with DIN 10113-1: 1997-07 provided results with up to 46 % higher pathogen recovery than the dry swabbing technique used. The different results of the various WDS methods show that the contact pressure of the swab and the moisture of the swab itself are important sampling factors that must be taken into account during sampling. With higher contact pressure, recoveries were on average 12 % higher than when lower contact pressure was applied. The swabs from the laboratory supply and the cosmetic swabs achieved comparable results, so that the use of commercially available cosmetic swabs is possible as long as contamination of the samples by prior sterilization is excluded.

Compliance with Ethical Standards

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