*hygiene*

MDPI

*Article*

# Comparison of Different Variants of Intermediate Cluster Disinfection

**Muriel Schulze, Franziska Nankemann, Anne Tellen, Julia Nitz, Yanchao Zhang, Stefanie Leimbach and Volker Krömker ***

Department of Microbiology, Faculty of Mechanical and Bioprocess Engineering, University of Applied Sciences and Arts, Heisterbergallee 10a, 30453 Hannover, Germany; muriel.schulze@tiho-hannover.de (M.S.); franziska.nankemann@hs-hannover.de (F.N.); anne.tellen@hs-hannover.de (A.T.); julia.nitz@hs-hannover.de (J.N.); yanchao.zhang@hs-hannover.de (Y.Z.); stefanie.leimbach@hs-hannover.de (S.L.)

* Correspondence: volker.kroemker@hs-hannover.de

**Abstract:** Intermediate cluster disinfection plays a vital role in preventing the transmission of mastitis pathogens during milking. This study evaluates the efficacy of different disinfection methods on teat liners through a randomized controlled field trial conducted on three dairy farms in Germany. The treatments assessed included dipping the liners in water, peracetic acid solution (PAS, 0.1%), and plasma-activated buffered solution (PABS). Total bacterial count (TBC) and the bacterial load of presumptive *Staphylococcus (S.) aureus* were measured using the wet–dry swab method. The results showed that PAS significantly reduced both TBC and *S. aureus* by 90% and 99%, respectively. PABS also demonstrated a significant reduction in the bacterial load of *S. aureus*. In contrast, dipping in water had no significant effect on either TBC or *S. aureus* bacterial counts. This study suggests that while PAS is the more effective disinfectant, PABS may offer an alternative with some antimicrobial activity. Further research on intermediate cluster disinfection in general is necessary to optimize its application and assess its long-term effectiveness in dairy farming practices.

**Keywords:** intermediate cluster disinfection; plasma-activated buffered solution; peracetic acid solution

check for updates

Academic Editor: Fernando Pérez-Rodríguez

Received: 19 January 2025
Revised: 23 February 2025
Accepted: 13 March 2025
Published: 17 March 2025

**Citation:** Schulze, M.; Nankemann, F.; Tellen, A.; Nitz, J.; Zhang, Y.; Leimbach, S.; Krömker, V. Comparison of Different Variants of Intermediate Cluster Disinfection. *Hygiene* **2025**, *5*, 12. https://doi.org/10.3390/hygiene5010012



## 1. Introduction

Modern udder health management involves different hygienic measures, especially in the field of housing hygiene and milking hygiene [1]. Even though the etiology of mastitis is complex, the transmission of mastitis pathogens between cows can occur during milking. One potential mode of transmission can be attributed to the contamination of the teat skin or teat canal during the milking process [2]. This indicates that cow-associated pathogens can be transmitted via the liner, as they are in direct contact with the teat. The most relevant pathogens implicated in this context are *Streptococcus agalactiae* and *Staphylococcus (S.) aureus*, which can be isolated from milk, the hands of the milker, milking gloves, or the liner during milking [3,4]. Therefore, the implementation of intermediate disinfection measures for milking equipment is a recommended strategy for the prevention of mastitis pathogen transmission during the milking process [5,6].

For intermediate disinfection, the following methods can be used: dipping the cluster into a bucket, spraying the liners, and utilizing a variety of commercially available disinfection systems, including backflush systems [7]. On farms in Germany, where an intermediate cluster disinfection is common practice, mostly a 0.1% peracetic acid solution (PAS) is used as the disinfectant. In automatic milking systems, the disinfectant solutions that are currently available contain mixtures comprising hydrogen peroxide, peracetic acid, and acetic acid to reduce corrosivity. Solutions containing PAS have good bactericidal activity, a broad spectrum of activity, and are effective over a wide temperature range [8,9]. A further advantage is that no residues remain on the material due to the rapid dismantling of the substance, thereby eliminating waiting periods [10]. In general, PAS must be diluted with water to prevent damage to mucous membranes, eyes, and lungs [9].

The use of plasma-activated liquids (PALs), including plasma-activated water and plasma-activated buffered solutions (PABS), could represent an environmentally friendly, non-thermal, and non-toxic alternative to traditional disinfectants. During the production process, the reaction of water or buffered solutions produces reactive oxygen and nitrogen species (RONS), which show significant biochemical activity. For microbial inactivation, the transfer and solvation of RONS from the gas phase to the liquid phase are of high importance [11]. RONS include both long-lived species, such as nitrates, nitrites, hydrogen peroxide, and ozone, and short-lived radicals, including hydroxyl radicals and atomic oxygen [12]. Consequently, despite variations in their composition, PALs can remain effective for several days depending on storage conditions due to their residual antimicrobial properties [13]. While it is known that plasma-induced RONS can damage bacterial cell walls, proteins, and DNA [14], further research is needed to clarify which specific RONS play a key role in the antimicrobial effectiveness of PALs, thus enhancing the disinfection process [11]. The study conducted by Scheib et al. [15] investigated how spraying with water, PABS, and PAS influences the bacterial load on teat liners. In general, the reduction of bacterial load was not significant for PABS, which may be due to the small sample size and the relatively short application time of the disinfectant solution. Therefore, the aim of this study was to evaluate the disinfection efficacy of various intermediate cluster disinfectant solutions by conducting a field study on three farms where the liners were dipped in different disinfectant solutions for comparative analysis.

## 2. Materials and Methods

An application for a license for animal testing was not required because no animals were used in this experiment. This study was approved by the Animal Welfare Committee of the University of Veterinary Medicine Hannover, Foundation, Hanover, Germany (TVO-2023-V-57).

### *2.1. Farms*

A total of 60 milking clusters were sampled on three dairy farms in Northern Germany after milking on 10 June 2024. These farms included two conventional and one organic dairy farm, with herd sizes ranging from 220 to 600 animals, an annual average milk yield of 9000–14,000 L per animal, and a bulk tank milk cell count of 140,000–280,000 cells/mL (annual mean).

### *2.2. Preparation of Treatment Solutions*

PABS was produced utilizing a plasma source created by the Faculty of Engineering and Health (HAWK, University of Applied Sciences and Arts, Göttingen, Germany) based on the principle of a single-insulated dielectric barrier discharge. Tris(hydroxymethyl)aminomethane (TRIS, also known as Trometamol, 99.8%, VWR International GmbH, Darmstadt, Germany) and TRIS-HCl (TRIS(hydroxymethyl)aminomethane hydrochloride, 99.0%, VWR International GmbH) were treated with this plasma source for 40 min, yielding approximately 0.2 L of PABS with a neutral pH. The concentrations of nitrate anions ($NO_3$; around 7700 mg/L), nitrite anions ($NO_2$; approximately 1200 mg/L), and hydrogen peroxide ($H_2O_2$; about 2.7 mg/L) were measured using a Reflectoquant

(Merck KGaA, Darmstadt, Germany) in PABS. PABS was transported to the trial farms at room temperature within four hours of its preparation. The same concentration as in Scheib et al. [15] was used. PAS was prepared as a 0.1% solution shortly before usage. Tap water from the farms was used for treatment of the liners.

### 2.3. Sampling Method

Sampling was performed with the modified wet–dry swab method (WDS) in accordance with DIN 10113-1:1997-07. This methodology was also used in the study conducted by Scheib et al. [16] and has proven to be a suitable quantitative swab sampling method for milking liners. Sterile swabs and a sterile swab solution were used for sampling. One-quarter strength sterile Ringer's solution (Merck KGaA) was used for the swabs, with 3 mL per test tube, and 2.2% sodium thiosulfate (APPLICHEM GmbH, Darmstadt, Germany) and 0.2% catalase (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) were then added as disinhibitors to this solution. Two swabs were always used for one sample. The first swab was moistened by dipping it in the solution for 5 s. The excess liquid was squeezed out. After the swab had been used to wipe the surface of the liner, a second dry swab was used for the same surface. All swab samples were taken at 5 cm depth and one rotation of 360°. During this process, the swab itself rotated and touched the surface only once. The pressure was so strong that the wooden pin was bent. After taking both samples, the swabs were placed in the same test tube. To prevent contamination of the swab medium through the samplers, the handles were broken off as they entered the test tube. Sampling was performed by four different people who were either familiar with this type of sampling or had been trained in advance.

### 2.4. Sampling

The liners were randomly assigned different treatments by attaching a different-colored tape to each liner of the milking cluster. Each color corresponded to the application of a different treatment. The first liner was designated and directly sampled as the untreated control. The second liner was dipped in tap water for 30 s, and then allowed to drain for an additional 30 s. A sample was subsequently obtained. The third liner was treated with PAS (0.1%) in accordance with the procedure and was then sampled. The fourth liner was treated with PABS and sampled. The assignment of teat liners to specific treatments was blinded from the laboratory diagnostics team.

### 2.5. Microbiological Examination

The test tubes were shaken before serial dilution was performed by using method L 00.00-54 of §64 LFGB (German Foods, Consumer Goods, and Foodstuffs Code). Following this, the samples were thoroughly mixed again before being spread at several dilution steps ($10^{-1}$ and $10^{-2}$) in duplicate onto Plate Count Skim Milk Agar (Carl Roth GmbH & Co. KG) for the determination of the total bacterial count (TBC), and Baird Parker Agar for the detection of presumptive *S. aureus*. The incubation of the Plate Count Skim Milk Agar was carried out at 30 °C for 24 h, while the Baird Parker Agar plates were incubated at 37 °C and examined after 24 and 48 h.

### 2.6. Statistics

Data were collected and analyzed by using the SPSS 29.0 program, SPSS Inc. (Chicago, IL, USA) in Microsoft Excel. The outcome variables, TBC and presumptive *S. aureus* cfu/cm$^2$, were transformed by applying log10 to approximate a normal distribution. The influence of the fixed factor treatment (water vs. PAS vs. PABS vs. control) on the bacterial load on the teat liner surface was analyzed using a linear mixed model. Estimated mean values were calculated, and a post hoc analysis was performed using Fisher's least significant difference (LSD) test with the Bonferroni correction. Statistical significance was defined at $p < 0.05$.

## 3. Results

In this study, 60 milking clusters from three different farms were sampled directly after milking on 10 June 2024. For each cluster, one liner served as the control, the second was dipped in water, the third was dipped in PAS (0.1%), and the fourth was dipped in PABS (same concentration as in Scheib et al. [15]).

### 3.1. Effect of the Treatments—Total Bacterial Count (TBC)

The analysis of the bacterial load of TBC (Table 1) showed that the treatment with PAS resulted in a significant reduction in bacterial counts on the teat liner by almost one log level. In contrast, water and PABS did not significantly differ from the control (Table 2).

**Table 1.** Descriptive means of the bacterial load of total bacterial count on the teat liner in $\log_{10}$ cfu/mL.

| Treatment | Mean | Std. Deviation [3] |
|---|---|---|
| Control | 4.3 | 0.4 |
| Water | 4.3 | 0.3 |
| PABS [1] | 4.2 | 0.4 |
| PAS [2] | 3.3 | 1 |

[1] plasma-activated buffered solutions, [2] peracetic acid solutions, and [3] standard deviation.

**Table 2.** Estimated means of the bacterial load of total bacterial count on liners in $\log_{10}$ cfu/mL.

| Treatment ($T_1$) | Treatment ($T_2$) | Estimated Means of Treatment ($T_1$) | Mean Difference ($T_1$–$T_2$) | Std. Error ($T_1$) [3] | *p*-Level |
|---|---|---|---|---|---|
| control | PABS | 4.2 | 0.1 | 0.3 | 0.6 |
| | water | | −0.1 | | 0.5 |
| | PAS | | 0.9 * | | <0.001 |
| PABS [1] | control | 4.1 | −0.2 | 0.3 | 0.6 |
| | water | | −0.9 | | 0.3 |
| | PAS | | 0.8 * | | <0.001 |
| water | control | 4.3 | 0.1 | 0.3 | 0.5 |
| | PABS | | 0.2 | | 0.3 |
| | PAS | | 1.0 * | | <0.001 |
| PAS [2] | control | 3.3 | −0.9 * | 0.3 | <0.001 |
| | PABS | | −0.8 * | | <0.001 |
| | water | | −1.0 * | | <0.001 |

[1] plasma-activated buffered solutions, [2] peracetic acid solutions, and [3] standard error. * the mean difference is significant.

### 3.2. Effect of the Treatments—Bacterial Load of Presumptive *Staphylococcus aureus*

The analysis of the bacterial load of presumptive *S. aureus* (Table 3) showed that the treatment with water resulted in no reduction in the bacteria load with 2.5 log10 cfu/mL. The treatment with PABS led to a significant reduction of 0.3 log10/$cfu^2$ of presumptive *S. aureus* in comparison to the control. The treatment with PAS showed a significant reduction in the bacterial counts on the teat liner from 2.4 $\log_{10}$ cfu/mL to 0.9 $\log_{10}$ cfu/mL (Table 4).

**Table 3.** Descriptive means of the bacterial load of *S. aureus* on liners in $\log_{10}$ cfu/mL.

| Treatment | Mean | Std. Deviation [3] |
|---|---|---|
| Control | 2.5 | 0.7 |
| Water | 2.5 | 0.6 |
| PABS [1] | 2.1 | 0.7 |
| PAS [2] | 1 | 0.9 |

[1] plasma-activated buffered solutions, [2] peracetic acid solutions, and [3] standard deviation.

**Table 4.** Estimated means of the bacterial load of *S. aureus* on liners in $\log_{10}$ cfu/mL.

| Treatment ($T_1$) | Treatment ($T_2$) | Estimated Means of $T_1$ | Mean Difference ($T_1$–$T_2$) | Std. Error ($T_1$) [3] | *p*-Level |
|---|---|---|---|---|---|
| control | PABS | 2.4 | 0.3 * | 0.14 | 0.007 |
| | water | | −0.1 | | 0.878 |
| | PAS | | 1.5 * | | <0.001 |
| PABS [1] | control | 2.1 | −0.3 * | 0.14 | 0.007 |
| | water | | −0.4 * | | 0.004 |
| | PAS | | 1.2 * | | <0.001 |
| water | control | 2.5 | 0.1 | 0.14 | 0.878 |
| | PABS | | 0.3 * | | 0.004 |
| | PAS | | 1.6 * | | <0.001 |
| PAS [2] | control | 0.9 | −1.5 * | 0.14 | <0.001 |
| | PABS | | −1.2 * | | <0.001 |
| | water | | −1.6 * | | <0.001 |

[1] plasma-activated buffered solutions, [2] peracetic acid solutions, and [3] standard error. * the mean difference is significant.

## 4. Discussion

A field study was carried out on three farms, where liners were dipped after milking in different solutions to compare the disinfecting effect on TBC and the bacterial load of presumptive *S. aureus* relative to a non-treated positive control. The quantitative detection of surface microbial counts on the liner surface is essential for assessing the effectiveness of intermediate disinfection in reducing bacteria. By measuring the microbial load on untreated and treated liners, we determined the extent of bacterial reduction achieved through the treatment process. This quantitative approach could provide a clearer and more objective evaluation of the disinfection efficacy of PAS and PABS. Furthermore, the liners were dipped in water to assess the mechanical rinsing effect on microbial counts. Therefore, the quantitative wet–dry swab method (WDS), in accordance with DIN 10113-1:1997-07, was used. It has been previously established that the WDS method is practical, fast, accurate, and repeatable. Recent studies have demonstrated that sponge bags are highly efficient as surface swabs, especially in the food industry [17]. However, they were not suitable for our study due to the specific structure and shape of the milking liners, which made swab sampling a more practical and effective choice in this context. The WDS method offers the advantage of a two-step process, in which an initial wet swab is applied first, followed by a dry swab. This approach enhances the detection of adhering pathogens on the liner. Given the relatively low bacterial load on the sampled surface, this method ensures the detection of even small reductions in bacterial counts. Additionally, the dry swab helps detect residual bacteria that remain after the wet swabbing step, further improving the accuracy of microbial assessment [15,18–20].

In this experiment, the sampling process involved four samplers, who each sampled an equal number of clusters, ensuring that the number of liners per treatment sampled was consistent between all participants. The results indicated no significant differences between the quarters or the samplers, demonstrating that the sampling method is standardized for use by various individuals. To effectively neutralize any bactericidal effects from disinfectant residues present in the swab medium post-sampling, a stop solution composed of sodium thiosulphate and catalase was incorporated to cancel the disinfection effect. This step is crucial for accurately assessing the impact of the disinfectant on the liner surfaces and for preventing false negative results [21].

We chose water as a comparison, as it is still common on many farms for milking clusters to be rinsed with potable tap water. The treatment with water showed no reduction in the TBC or presumptive *S. aureus*. In contrast, Skarbye et al. [22] demonstrated that rinsing the liner with 966 mL of water achieved complete elimination of *S. aureus*. This discrepancy may be attributed to differences in application methods, as their study used a rinsing process with a larger volume of water, whereas our study used dipping. These findings suggest that water may only be effective as a mechanical treatment when used in larger volumes and applied through rinsing.

For TBC and presumptive *S. aureus* loads, our results showed that treatment with PAS led to a significant reduction in bacterial loads of 90% and 99%, respectively, further supporting its efficacy as previously reported [8–10]. In contrast, Scheib et al. [15] found no significant reduction in bacterial counts with PAS treatment. Their study used spraying instead of dipping the liners, suggesting that the application method may influence the outcome. In this investigation, the liners were immersed in the disinfectant solution, which probably contributed to the observed differences. Unlike PAS, dipping the liners in PABS did not result in a significant reduction in TBC. A numerical reduction in TBC on liners was observed. However, it is likely that the sample size was too small to demonstrate a significant effect. Nevertheless, a significant reduction in the bacterial count of presumptive *S. aureus* was observed following treatment with PABS. On the one hand, the high resistance of S. aureus to PABS was previously demonstrated by Große Peclum et al. [23]. On the other hand, the study by Scheib et al. [15] demonstrated that treatment with PABS resulted in a reduction of *S. aureus* by one logarithmic level. The key advantage of PABS lies in its environmentally friendly, non-toxic, and cost-effective properties [24]. PABS represents a possible alternative to traditional chemical disinfectants, as it does not rely on chemical additives for energy transfer or chemical reactivity [25]. Furthermore, it is free of hazardous substances, unlike conventional disinfectants that require dilution before use. However, the plasma activation process necessary for PABS production requires a substantial amount of electrical energy [11], partially offsetting its environmental benefits. PABS retains its disinfecting properties for up to 24 h when stored at 21 °C, owing to the stability of its RONS. Like peracetic acid-based disinfectants, PABS must be prepared daily and applied after each milking session. Despite its advantages, the production of PABS remains both energy- and time-intensive, presenting a challenge to its broader application. In summary, dipping the liners in PAS demonstrated significant effects on the TBC and the number of presumptive *S. aureus*. A significant reduction in the bacterial count of presumptive *S. aureus* was also observed following the treatment of liners with PABS. In contrast, dipping the liners in water showed no significant reduction in TBC or presumptive *S. aureus* in the liners compared to the untreated control liners. Therefore, the mechanical rinsing effect alone was not sufficient to significantly reduce the bacterial loads in this trial.

## 5. Conclusions

In this randomized field trial, the efficacy of different intermediate cluster disinfection solutions was assessed based on their effects on TBC and presumptive *S. aureus* load on teat liners after milking. Treatment with PABS resulted in a significant reduction in presumptive *S. aureus* counts (0.3 log10 cfu/mL). However, neither PABS nor water treatment achieved a significant reduction in TBC. PAS was shown to be an effective disinfectant, achieving a 90% reduction in TBC and nearly 99% in presumptive *S. aureus*. While PABS could be a potential alternative disinfectant, further research on intermediate cluster disinfection in general is necessary to optimize its application and assess its long-term effectiveness in dairy farming practices. Nonetheless, the results highlight the crucial importance of adding disinfecting substances when dipping the liners, as the treatment with drinking tap water alone showed no significant reduction in pathogen levels.

**Author Contributions:** Conceptualization, V.K.; methodology, V.K., M.S. and S.L.; validation, V.K.; formal analysis, V.K.; investigation, M.S., Y.Z., A.T., J.N., F.N., S.L. and V.K.; resources, Y.Z. and; data curation, V.K.; writing—original draft preparation, M.S.; writing—review and editing, F.N., S.L. and V.K.; visualization, M.S.; supervision, V.K.; project administration, V.K.; funding acquisition, S.L. and V.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the European Innovation Partnership (EIP-AGRI) project "Plasmadip" and funded by the European Agricultural Fund for Rural Development (EAFRD) and the Steinbeis Research Center Milk Science, Kirchlengern, Germany (2-24).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The raw data supporting the conclusions of this article will be made available by the authors on request.

**Acknowledgments:** We would like to thank the participating dairy farms and the Faculty of Engineering and Health (HAWK), University of Applied Sciences and Arts in Göttingen, Germany for providing the plasma-activated buffer solution.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References


1. Dufour, S.; Fréchette, A.; Barkema, H.W.; Mussell, A.; Scholl, D.T. Invited review: Effect of udder health management practices on herd somatic cell count. *J. Dairy Sci.* **2011**, *94*, 563–579. [CrossRef] [PubMed]
2. Hässig, M.; Sigrist, S.M.; Corti, S.; Giezendanner, N.; Stephan, R. The role of bacterial contamination of milking utensils and disinfecting solutions as a possible cause of clinical mastitis in dairy cows. *Schweiz. Arch. Tierheilkd.* **2011**, *153*, 263–268. [CrossRef] [PubMed]
3. De Visscher, A.; Supré, K.; Haesebrouck, F.; Zadoks, R.N.; Piessens, V.; Van Coillie, E.; Piepers, S.; De Vliegher, S. Further evidence for the existence of environmental and host-associated species of coagulase-negative staphylococci in dairy cattle. *Vet. Microbiol.* **2014**, *172*, 466–474. [CrossRef] [PubMed]
4. Woudstra, S.; Wente, N.; Zhang, Y.; Leimbach, S.; Kirkeby, C.; Gussmann, M.K.; Kromker, V. Reservoirs of *Staphylococcus* spp. and *Streptococcus* spp. Associated with Intramammary Infections of Dairy Cows. *Pathogens* **2023**, *12*, 699. [CrossRef]
5. Newbould, F.H.S.; Barnum, D.A. The reduction of the microflora of milking machine inflations by teat dipping and teat cup pasteurization. *J. Milk Food Technol.* **1960**, *23*, 374–376. [CrossRef]
6. Newbould, F.H. Disinfection in the prevention of udder infections: A review. *Can. Vet. J.* **1965**, *6*, 29–37.
7. Krömker, V. *Short Textbook Dairy Science and Milk Hygiene*; Parey: Stuttgart, Germany, 2006.
8. Kunigk, L.; Almeida, M.C. Action of peracetic acid on *Escherichia coli* and *Staphylococcus aureus* in suspension or settled on stainless steel surfaces. *Braz. J. Microbiol.* **2001**, *32*, 38–41. [CrossRef]
9. Bützer, P. Peroxyessigsäure: Einfach, aber wirksam (Peroxyacetic acid: Simple, but effective). *CLB Chem. Labor Biotech.* **2012**, *62*, 96–115.
10. Fröhling, A.; Wienke, M.; Rose-Meierhöfer, S.; Schlüter, O. Improved method for mastitis detection and evaluation of disinfectant efficiency during milking process. *Food Bioprocess Technol.* **2010**, *3*, 892–900. [CrossRef]
11. Laroussi, M.; Bekeschus, S.; Keidar, M.; Bogaerts, A.; Fridman, A.; Lu, X.; Ostrikov, K.; Hori, M.; Stapelmann, K.; Miller, V. Low-temperature plasma for biology, hygiene, and medicine: Perspective and roadmap. *IEEE Trans. Radiat. Plasma Med. Sci.* **2022**, *6*, 127–157. [CrossRef]
12. Hoeben, W.F.L.M.; van Ooij, P.P.; Schram, D.C.; Huiskamp, T.; Pemen, A.J.M.; Lukeš, P. On the Possibilities of Straightforward Characterization of Plasma Activated Water. *Plasma Chem. Plasma Process.* **2019**, *39*, 597–626. [CrossRef]

13. Traylor, M.J.; Pavlovich, M.J.; Karim, S.; Hait, P.; Sakiyama, Y.; Clark, D.S.; Graves, D.B. Long-term antibacterial efficacy of air plasma-activated water. *J. Phys. D Appl. Phys.* **2011**, *44*, 472001. [CrossRef]
14. Yusupov, M.; Bogaerts, A.; Huygh, S.; Snoeckx, R.; Van Duin, A.C.; Neyts, E.C. Plasma-induced destruction of bacterial cell wall components: A reactive molecular dynamics simulation. *J. Phys. Chem. C* **2013**, *117*, 5993–5998. [CrossRef]
15. Scheib, S.; Leimbach, S.; Avramidis, G.; Bellmann, M.; Nitz, J.; Ochs, C.; Tellen, A.; Wente, N.; Zhang, Y.; Viöl, W.; et al. Intermediate Cluster Disinfection: Which Disinfection Solution Is Most Effective on Milking Liners? A Comparison of Microorganism Reduction on Liner Inner Surfaces Using Quantitative Swab Sampling Technique. *Pathogens* **2023**, *12*, 560. [CrossRef]
16. Scheib, S.; Wente, N.; Leimbach, S.; Krömker, V. Comparison of different swab techniques for the quantitative analysis of mastitis relevant pathogens on liner surfaces. *Milk Sci. Int.-Milchwiss.* **2023**, *76*, 1–7. [CrossRef]
17. Faille, C.; Brauge, T.; Leleu, G.; Hanin, A.; Denis, C.; Midelet, G. Comparison of the performance of the biofilm sampling methods (swab, sponge, contact agar) in the recovery of Listeria monocytogenes populations considering the seafood environment conditions. *Int. J. Food Microbiol.* **2020**, *325*, 108626. [CrossRef]
18. Pfannenschmidt, F. Qualification of the Wet-Dry-Swab-Technique DIN 10113; 1997-07 for the Determination of the Hygienic Status in Milking Machines. Diss Stiftung Tierärztliche Hochschule Hannover. 2003. Available online: https://nbn-resolving.org/urn:nbn:de:gbv:95-87922 (accessed on 1 December 2003).
19. Paduch, J.-H.; Krömker, V. Besiedlung von Zitzenhaut und Zitzenkanal laktierender Milchrinder durch euterpathogene Mikroorganismen. *Tierärztliche Prax. Ausg. G Großtiere/Nutztiere* **2011**, *39*, 71–76. [CrossRef]
20. Hohmann, M.-F.; Wente, N.; Zhang, Y.; Klocke, D.; Krömker, V. Comparison of two teat skin sampling methods to quantify teat contamination. *Milk Sci. Int.-Milchwiss.* **2020**, *73*, 2–6.
21. Reybrouck, G. The testing of disinfectants. *Int. Biodeterior. Biodegrad.* **1998**, *41*, 269–272. [CrossRef]
22. Skarbye, A.P.; Krogh, M.A.; Denwood, M.; Bjerring, M.; Ostergaard, S. Effect of enhanced hygiene on transmission of Staphylococcus aureus, Streptococcus agalactiae, and Streptococcus dysgalactiae in dairy herds with automatic milking systems. *J. Dairy Sci.* **2021**, *104*, 7195–7209. [CrossRef]
23. Große-Peclum, V.; Siekmann, L.; Krischek, C.; Avramidis, G.; ten Bosch, L.; Harms, M.; Ochs, C.; Ortmann, R.; Hoedemaker, M.; Ahlfeld, B.; et al. An In Vitro Model Using TRIS-Buffered Plasma-Activated Water to Reduce Pathogenic Microorganisms Involved in Digital Dermatitis Infection in Cattle. *Appl. Sci.* **2022**, *12*, 12325. [CrossRef]
24. Rahman, M.; Hasan, M.S.; Islam, R.; Rana, R.; Sayem, A.; Sad, M.A.A.; Matin, A.; Raposo, A.; Zandonadi, R.P.; Han, H. Plasma-activated water for food safety and quality: A review of recent developments. *Int. J. Environ. Res. Public Health* **2022**, *19*, 6630. [CrossRef] [PubMed]
25. Zhou, R.; Zhou, R.; Wang, P.; Xian, Y.; Mai-Prochnow, A.; Lu, X.; Cullen, P.J.; Ostrikov, K.; Bazaka, K. Plasma-activated water: Generation, origin of reactive species and biological applications. *J. Phys. D Appl. Phys.* **2020**, *53*, 303001. [CrossRef]