Can a humid storage environment of surgical instruments before reprocessing increase patient safety and durability of instruments?

Rubak, Peter; Lorenzen, Jan; Ripadal, Krister; Christensen, Ann-Eva; Aaen, Dorthe; Nielsen, Hans Linde; Bundgaard, Karin

Published in:
Journal of Hospital Infection

DOI (link to publication from Publisher):
10.1016/j.jhin.2022.01.012

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

Journal of Hospital Infection 122 (2022) 64–71

Available online at www.sciencedirect.com

Journal of Hospital Infection

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

Can a humid storage environment of surgical instruments before reprocessing increase patient safety and durability of instruments?

P. Rubak [a], J. Lorenzen [b], K. Ripadal [c], A-E. Christensen [d], D. Aaen [e], H.L. Nielsen [e,f], K. Bundgaard [f,g,*]

[a] *Clinic for Diagnostics & Clinical Nursing Research Unit, Aalborg University Hospital, Aalborg, Denmark*
[b] *Danish Technological Institute, Aarhus, Denmark*
[c] *Clinic for Diagnostics, Aalborg University Hospital, Aalborg, Denmark*
[d] *Unit for Psychiatric Research, North Denmark Region, Aalborg, Denmark*
[e] *Department of Clinical Microbiology, Aalborg University Hospital, Aalborg, Denmark*
[f] *Department of Clinical Medicine, Aalborg University, Aalborg, Denmark*
[g] *Clinic for Neuro-, Head and Orthopaedic Diseases & Clinical Nursing Research Unit, Aalborg University Hospital, Aalborg, Denmark*

ARTICLE INFO

Article history:
Received 21 September 2021
Accepted 14 January 2022
Available online 22 January 2022

Keywords:
Reprocessing
Surgical instruments
Enterococcus faecalis ATCC 29212
Storage environment
Protein residue
Corrosion

SUMMARY

Background: National and international guidelines recommend reprocessing of medical instruments to commence as soon as possible post-surgery; furthermore, they recommend that transport and storage of surgical instruments postoperatively occurs in a moist, humid atmosphere. The concern is that a dry storage environment results in deterioration of instruments.

Aim: To evaluate whether residual protein or corrosion is associated with storage environment (dry or humid), holding time or number of treatment cycles.

Methods: The range of protein residue and corrosion were tested on surgical instruments contaminated with human blood amended *Enterococcus faecalis* ATCC 29212. Subsequently instruments were stored for 6, 12 and 24 h in dry or humid conditions. After one, 25 and 50 reprocessing cycles, instruments were examined for protein residues using the o-phthaldialdehyde (OPA) method or corrosion using stereomicroscopy, scanning electron microscopy and energy dispersive spectroscopy.

Findings: Protein residue found on instruments was 21.5–54.0 μg and corrosion corresponded to 0–5% of the inspected area. No associations between storage environment and protein residue (adjusted mean difference = 0.48, 95% confidence interval: -0.42, 1.37, P=0.30) or corrosion (P=0.20) were identified. Higher numbers of treatment cycles showed higher amounts of corrosion (mean: 1_{cycle} = 0.06%, 25_{cycles} = 0.52% and 50_{cycles} = 1.45%). In contrast, higher numbers of treatment cycles showed lower amounts of protein residue (P<0.001). We found both lower protein residue concentration and lower corrosion rating at 12 h compared with 6 and 24 h holding time.

* Corresponding author. Address: Sønderskovvej 5, 3rd floor, 9000 Aalborg, Denmark. Tel.: +45 23 49 75 19.
E-mail address: karin.mikkelsen@rn.dk (K. Bundgaard).

https://doi.org/10.1016/j.jhin.2022.01.012

Conclusion: Cleanliness and durability of instruments before reprocessing seems not to be affected by storage environment or holding time but instead by number of treatment cycles.

Introduction

According to national and international guidelines for infection control, reprocessing of reusable medical equipment is recommended to commence as soon as possible [1—3]. Furthermore, it is recommended that surgical instruments are transported and stored in a humid environment until reprocessing is commenced [1—3]. These recommendations are based on expert opinions, scientific literature, legal requirements and 'best practice'. However, evidence-based knowledge from systematic research is strongly warranted [1,2].

Reprocessing is recommended to start immediately after surgery, by removing visible contamination by wiping with a damp cloth and flushing cavities [1,2]. The aim is to prevent drying of bio-contamination, such as blood and tissue residues from the operation, on the instrument surface. In the drying process when liquid evaporates, salts residues from the organic material remains on the surface of the instruments. The drying time is the most critical factor in influencing the quality of instruments. The primary concern is the risk of corrosion and thereby destruction of the instrument surface. Several studies indicate that drying times beyond 15 min significantly reduces the effect of subsequent cleaning to remove protein residues, including prions. Furthermore, storing instruments in a humid environment directly after use may reduce the amount of protein/amyloid adsorption and enhance the effect of reprocessing [4—6].

When instruments are exposed to high temperatures associated with washing, disinfection and sterilization, and if cleaning is ineffective, the bio-contamination can, over time solidify in an insoluble biofilm that acts as a physical barrier that compromises the sterilization process and thereby threaten patient safety [7]. Biofilm consists of a thin layer of micro-organisms that, together with the microbial polysaccharides adhere to surfaces [8]. Once a biofilm is formed and allowed to dry, it is difficult to remove even with conventional automated cleaning methods due to its adhesion to the surface. At the same time, biofilm presence is difficult to identify as there will only be a small number of active cells present on the surface [9]. Costa *et al.*, who demonstrated the presence of biofilm on six of seven discarded instruments, support this; however, no viable bacteria were detected after steam sterilization [7].

Research supporting the recommendation for the storage of instruments in a humid environment between theatres to reprocessing sites is sparse and no research to date has been identified that describes how transport and storage environment influence the occurrence of corrosion. Therefore, the aim of the study was to test the hypothesis that storing post-surgical instruments in a humid environment until reprocessing reduces the occurrence of corrosion, as well as the occurrence and accumulation of biological material compared with instruments stored in a dry environment.

Methods

To match real-life, both simple instruments such as forceps and more complex instruments with cavities such as irrigation needles were investigated. Forceps and needles were produced of stainless steel in accordance with ISO 7153-1 [10]. Furthermore, a solution of human blood and *E. faecalis* ATCC 29212 (final concentration: 1.5×10^8 cfu/mL) was used for contamination to match real-life soiling in an operating site.

Contamination was measured on 108 irrigation needles and 108 forceps. The forceps were contaminated by opening and closing them five times in a solution of human blood containing *E. faecalis* and the needles were flushed with the solution five times. Subsequently the instruments were stored for 6, 12 and 24 h at room temperature before reprocessing. Half of the items were stored dry and the other half in a humid environment. Instruments were re-contaminated before each reprocessing cycle. Each instrument had the same holding time and storage environment for up to 50 reprocessing cycles. After one, 25 and 50 cycles, six instruments of each type, holding time and storage environment were removed after washing, but before disinfection, and examined for contamination, i.e., six instruments for each measuring point. One positive and one negative control were also included in the study for each instrument type. The positive control was contaminated but not washed and the negative control was washed but not contaminated. Contamination from bacteria and blood was measured as protein residue using the o-phthaldialdehyde (OPA) method [11].

The same procedure was followed when testing for corrosion, where 108 forceps were contaminated in a similar solution of human blood containing *E. faecalis* and stored for 6, 12 and 24 h at room temperature before reprocessing. Respectively, 54 forceps were stored in an open and dry environment and 54 forceps were stored in a closed humid environment. After one, 25 and 50 reprocessing cycles, six instruments of each holding time and storage environment were examined for corrosion by visual inspection (stereomicroscopy) and scanning electron microscopy (SEM) [12].

All instruments were washed in the washer-disinfector using the standard protocol for the Sterile Centre at Aalborg University Hospital, Denmark (see Supplementary data).

No clear definition of how to create a humid environment for transport and storage of instruments between theatres and reprocessing sites exists today. Some theatres imitate a humid environment by using transport boxes covered with lightly humidified cotton gauze, while others immerse instruments in water with added detergent or spray them with various presoaking products. In order to achieve reliability in this project, the humid transport and storage environment was the same throughout the trial period. This was accomplished by covering instruments in each closed transport box with the same amount of cotton gauze wetted with the same amount of

sterile water. Dry environment is defined as instruments packed in open transport boxes without cover.

OPA analysis

The OPA analysis was based on EN-ISO 15883-1: 2009 [11]. The forceps were eluted with 10 mL 1% SDS in stomacher bags. Items were sonicated at 40 kHz for 5 min at 30–35 °C in a degassed sonication bath (Branson 2800). The items then rested for 20 min at 23 °C, after which the eluate was transferred to 15-mL pipes and exposed to vortexing for 5 s prior to transfer of 3 × 100 μL to a 96-well microtiter plate.

The irrigation needles were also eluted in 10 mL 1% SDS. The needles were rinsed using a 3-mL syringe, filling and emptying it five times. Items were placed in sterile stomacher bags and sonicated at 40 kHz for 5 min at 30–35 °C (Branson 2800). The items then rested for 20 min at 23 °C, after which they were rinsed additionally five times. Finally, the eluate was transferred to 15-mL pipes and exposed to vortexing for 5 s prior to transfer of 3 × 100 μL to a 96-well microtiter plate. One dummy, consisting of 10 mL 1% SDS in a stomacher bag processed the same way as the test instruments, was also included in the analysis. In Denmark, the consensus acceptable level for surface protein residues is a maximum of 100 μg/instrument [13].

Corrosion analysis

The manufacturer reported the surgical forceps to have a chromium content of 12–14%.

The visual inspection included examining each forceps gripping area on both sides at 25× magnification. Detected corrosion and other defects such as discolouration or mechanical damage were documented through imaging at a representative magnification. The surface of each forceps was examined in three areas using SEM. All three areas were examined with a magnification of 500× for irregularities or corrosion attacks. For each area, a section was also examined using a 2000× magnification (Keyence VHX-6000). Irregularities or corrosion were examined with an appropriate magnification or with chemical analysis using energy dispersive spectroscopy (EDS) [12]. The degree of corrosion was assessed according to the DS/EN ISO 10289:2001 and each corroded area was measured and summed up to calculate the percentage of area of defects (Table I) [12].

The Danish Technological Institute conducted the protein residue and corrosion analyses.

Data analysis

The association between storage environment and protein residue was analysed using linear regression, adjusted for type of instrument, number of cycles and holding time. Sensitivity analyses using bootstrap as well as Mann–Whitney U-test were carried out in case of non-normality and heteroscedasticity. Further, the amount of protein residue was compared for one, 25 and 50 cycles as well as for 6, 12 and 24 h, respectively, using linear regression, adjusted for type of instrument, storage environment as well as number of cycles or holding time as appropriate. A boxplot was provided to visualize the distribution of protein residue and of ratings for corrosion. The latter was then compared using Fishers exact test between dry and humid storage environment, one, 25 and 50 cycles as well as for 6, 12 and 24 h of holding time, respectively.

Table I
Corrosion adjustment scale

Area of corrosion, A (%)	Rating, Rp (-)
No defects*	10
0 <A ≤0.1	9
0.1 <A ≤0.25	8
0.25 <A ≤0.5	7
0.5 <A ≤1.0	6
1.0 <A ≤2.5	5
2.5 <A ≤5.0	4
5.0 <A ≤10	3
10 <A ≤25	2
25 <A ≤50	1
50 <A	0

* According to DS/EN ISO 10289:2001 if A ≤0.046416% then the haemostat is rated as a 10.

Results

Twenty-five and 50 reprocessing cycles were performed, however, a tight schedule at the reprocessing site resulted in a reduced number of cycles ranging from 21 to 24 cycles for instruments planned for 25 cycles, and 46–49 cycles for instruments planned for 50 reprocessing cycles. This caused some instruments to have a longer holding time than planned, however the prolonged holding time was the same for both storage environments. The room temperature in the Sterile Centre during the trial fluctuated from 22.3 to 24.8 °C, with the lowest temperatures on Mondays and the highest temperatures on Fridays. Humidity in the room where instruments were stored dry ranged from 9% to 55.1% (mean: 26.7%, standard deviation (SD): 10.8%). Humidity in humid storage increased from 92.8% to 95.2% (15 min–24 h). The gauze was equally wet after 6, 12 and 24 h, where no difference in weight was observed. Furthermore, instruments with holding times of 6, 12 and 24 h were packed separately, and the boxes remained closed until reprocessing.

Protein residue

In total, protein residue from 108 irrigation needles and 108 forceps was quantified using the OPA method. Each 96-well microtiter plate contained eluate from 18 instruments, two controls (negative and positive) and two blanks (dummy and 1% SDS). All samples were analysed in triplicate.

Protein residue concentration was calculated using linear regression based on a standard series of nine different concentrations. Protein residue on the instruments was in general low. Across storage environment, holding time and number of reprocessing cycles, protein residue ranged from 21.8 to 28.1 μg (mean: 24.4 μg, SD: 1.3 μg) on the forceps and 21.5 to 54.0 μg (mean: 26.7 μg, SD: 4.9 μg) on the needles.

The negative control showed low levels of protein residue (mean: 24.5 μg, SD: 1.8 μg, $N = 2$) below or comparable to the test instruments (Figure 1) whereas the positive control showed a high degree of protein residue (mean: 2899.2 μg, SD:

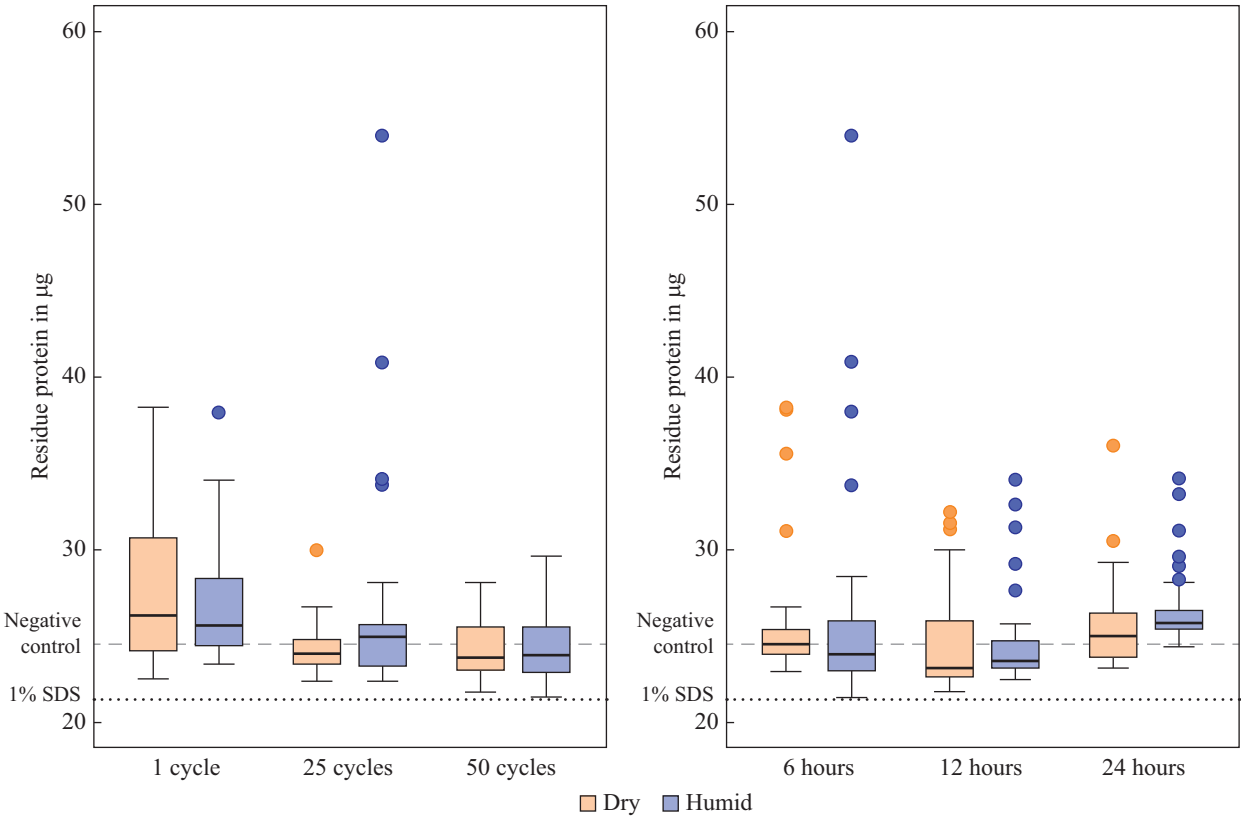

Figure 1. Protein residues on forceps and needles combined for number of reprocessing cycles (left) or holding time (right) visualized for dry and humid storage environment.

380 µg, $N = 2$). Both 1% SDS (mean: 21.3 µg, $N = 1$) and the dummy (mean: 22.2 µg, $N = 1$) showed lower levels of protein residue compared with the negative control. Mean concentration of protein residue was found to be 25.3 µg (SD: 3.2 µg) and 25.8 µg (SD: 4.3 µg) for dry and humid storage environment, respectively. No association between storage environment and the amount of protein residue was found (Table II).

Distribution of protein residue for reprocessing cycles and holding time were visualized in Figure 1. We found a negative association and a statistically significant difference in amount of protein residues when comparing one, 25 and 50 cycles (Table II; Figure 1, left). A different pattern was seen for holding time and protein residue, where a lower mean protein concentration was observed at 12 h for both dry and humid storage environment compared with 6 and 24 h (Table II; Figure 1, right).

Corrosion

In total, 108 forceps were analysed and rated using visual inspection, SEM and EDS. Stereomicroscopy showed areas with corrosion corresponding to 0–5% of the investigated surface area. The forceps with one reprocessing cycle were used as reference instruments. The surface examination of the forceps using SEM revealed pitting corrosion in random areas, these results were not involved in the overall corrosion rating. In several of the analysed locations, EDS revealed particles mostly consisting of silicon (Si), calcium (Ca) and/or aluminium (Al). These particles were evaluated to be present due to the metal composition of the instruments and not caused by corrosion. Dark spots on the instrument surfaces were also observed by electron microscopy. EDS showed that these spots contained high amounts of carbon (C) or sodium (Na) and chlorine (Cl). The dark spots were assessed to originate from the contamination or sterilization procedure and hence not as results of corrosion attacks.

No statistically significant difference was found in the distribution of corrosion ratings for dry compared with humid storage environments (P=0.20). Stereomicroscopy inspection of instruments showed corrosion ranging from 0 to 0.25% (mean: 0.06%), 0.25 to 5.0% (mean: 0.52%) and 0.25 to 5.0% (mean: 1.45%) of the inspected surface area for one, 25 and 50 cycles, respectively. We found a negative association between occurrence of corrosion and number of cycles (P<0.001). The same association was found for both environments (Figure 2, left). We found no association between holding time and the occurrence of corrosion (P=0.47). Ratings in the two environments followed the same pattern (Figure 2, right).

When investigated at high magnification, forceps showed defects around the area of the teeth as illustrated in Figure 3. However, these defects were observed on instruments across all reprocessing cycles.

Discussion

National and international guidelines for reprocessing of sterilizable medical equipment recommend that surgical instruments are transported and stored in a humid environment

Table II
Associations and differences between storage environment, reprocessing cycles and holding time and the amount of protein residue

Storage environment					
	Dry	Humid		*P* (Wald*)	*N*
Crude mean difference (CI)	Ref**	0.48 (-0.54, 1.49)		0.36	216
Adjusted*** mean difference (CI)	Ref	0.48 (-0.42, 1.37)		0.30	216
Reprocessing cycles					
	1 Cycle	25 cycles	50 cycles	*P* (Wald)	*N*
Crude mean difference (CI)	Ref	-1.86 (-3.03, -0.68)	-2.94 (-4.11, -1.76)	<0.001	216
Adjusted mean difference (CI)	Ref	-1.86 (-2.96, -0.75)	-2.94 (-4.04, -1.83)	<0.001	216
Holding time					
	6 h	12 h	24 h	*P* (Wald)	*N*
Crude mean difference (CI)	Ref	-1.46 (-2.69, -0.24)	0.05 (-1.17, 1.27)	0.023	216
Adjusted mean difference (CI)	Ref	-1.46 (-2.56, -0.36)	0.05 (-1.05, 1.15)	<0.001	216

CI, confidence interval.
* *P*-value was calculated using Wald test.
** Reference group.
***The calculation was adjusted for instrument type, environment, reprocessing cycles and holding time as appropriate.

[1,2] due to the concern that contamination may dry out and thereby increase the risk of corrosion and destruction of the instrument surface. However, based on the findings in this study we challenge this recommendation. Our results found no association between either storage environment and the amount of protein residue, or between storage environment and corrosion on any of the tested instruments.

We saw fluctuations in temperature and humidity during the test period, however these are not considered relevant to the results because they were the same across holding time, number of cycles and storage environment. In our study the humid environment was created by covering test instruments in sterile water moistened gauze and storing them in closed containers, thus the humid environment was comparable

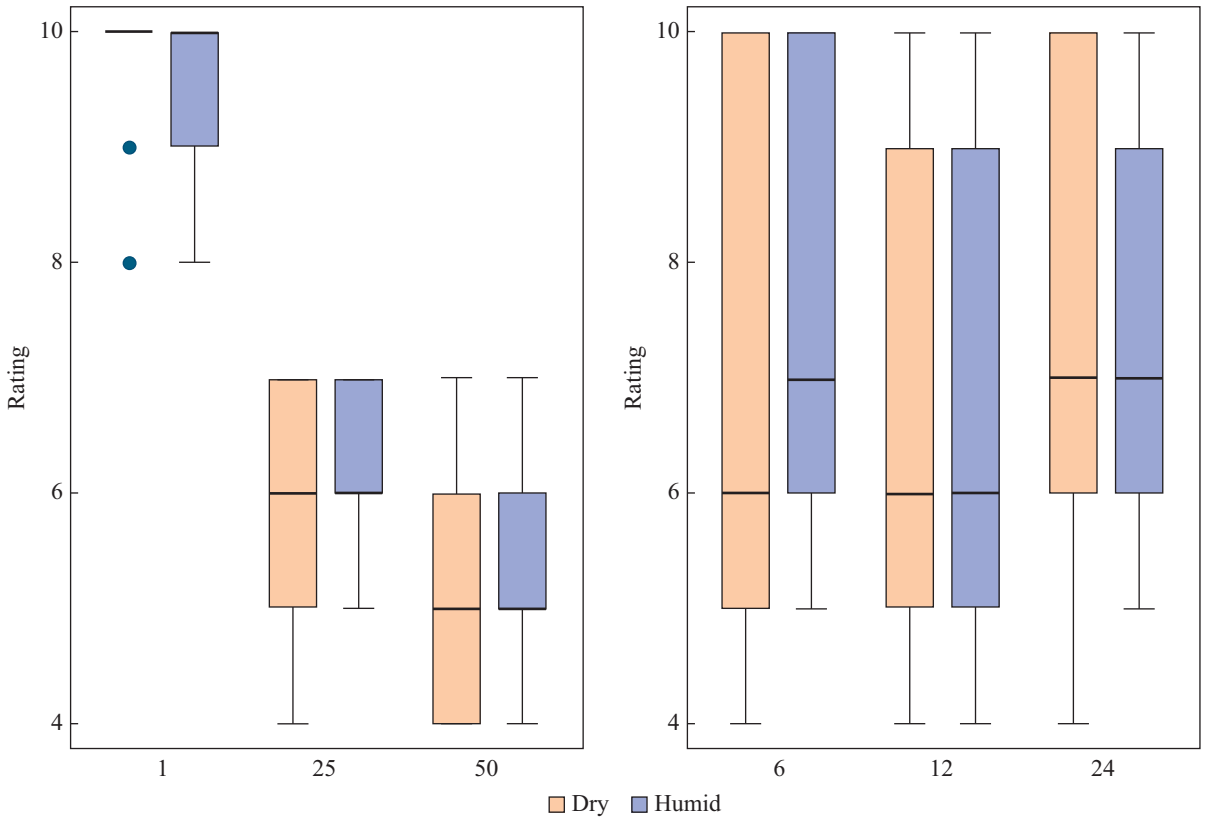

Figure 2. Corrosion rating and number of reprocessing cycles (left) and corrosion rating and holding time (right) visualized for dry and humid storage environment.

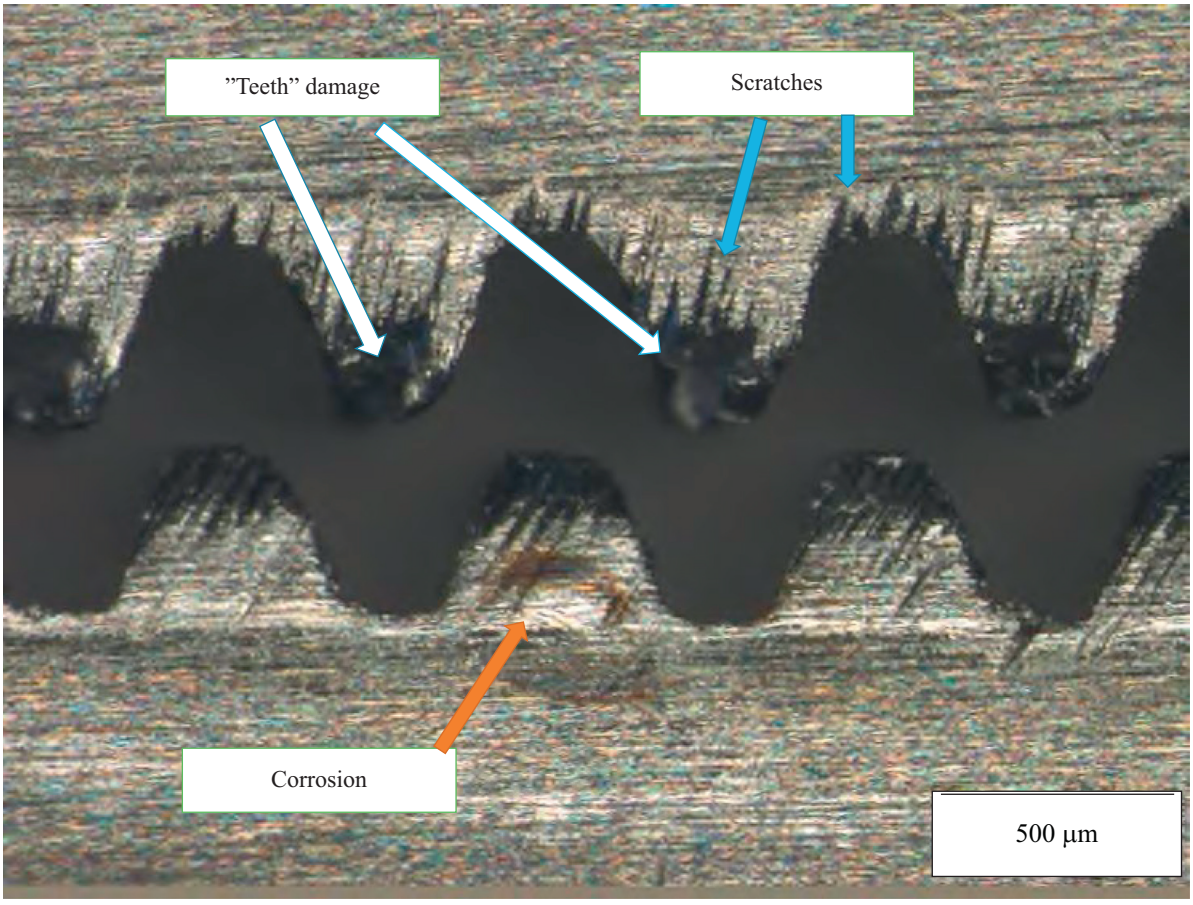

Figure 3. Example showing mechanical damage and corrosion. Forceps stored in a humid environment, 6 h holding time and 25 reprocessing cycles. Photo: Danish Technological Institute, Aarhus, Denmark.

across holding time and number of cycles. However, one may question whether other forms of humidified environments such as, for example, choosing an enzymatic pre-soaking agent would have affected the result? The effects of using pre-soaking agents were examined by Aasim *et al.* [14], who showed that pre-soaking in an enzymatic cleaner prior to ultrasonic cleaning had no significant effect on the cleanliness of endodontic files. In comparison, Lipscomb *et al.* demonstrated an up to 96% reduction in prion-infected tissue contamination after application of different commercially available pre-soak solutions [4]. The different results may be connected to what kind of soiling the instruments have been submitted to. In our study the reference strain of *E. faecalis* commonly used in laboratory studies was chosen because of its well documented ability to form biofilm, and in connection with the human blood, the soiling is comparable to real-life circumstances in an operating site [15]. In this study, we examined instruments after they were washed and not before as in, for example, Percin *et al.* [16] and Mohite *et al.* [17] where a bacterial count was included. As a result of the quality of the washing procedures, we considered it unrealistic to detect any viable bacteria after washing. We amended the human blood with bacteria as we expected they would contribute to an increased soil adhesion on the instrument surface. The instruments were then analysed for protein which was residue from both blood and bacteria. Lopes *et al.* found that once biofilm is formed and allowed to dry out on the instrument, it is difficult to remove even with conventional automated cleaning methods due to its adhesion to the surface [9]. In concordance, Roberts showed that biofilm formation can be controlled by prompt device cleaning and reprocessing [18]. Our study showed low or no difference in protein residues on instruments stored dry compared with instruments stored in a humid environment. This could be a result of the quality of our reprocessing procedures.

We detected statistically significant associations between holding time and protein residue, and between number of cycles and protein residue. However, these associations are not considered to be clinically relevant as even the highest concentration of protein residue of 54.0 μg is below the accepted threshold of 100 μg [12]. The increased concentration of protein residues on the instruments with one reprocessing cycle compared with 25 and 50 reprocessing cycles (Figure 1) may be explained by the fact that instruments with only one cycle along with the negative control were used directly from the manufactures packaging. Hence, protein residue from production would be added on top of the result from the contamination as the instruments with one reprocessing cycle were washed but not disinfected or sterilized before being contaminated [19]. However, the amount of protein residue

added from production would be low as the negative control was also washed but not disinfected or sterilized before entering the study (Figure 1). As a result, the difference between protein residue at one, 25 and 50 cycles may be overestimated. However, this has no effect on the overall conclusion, the results would thereby show no association compared to a declining association.

The findings of pitting corrosion in random areas and particles consisting of Si, Ca and/or Al embedded in the metal of the forceps across storage environment, holding time and number of reprocessing cycles are comparable to the findings by Bundgaard *et al*. [20]. Both pitting and embedding of particles in the surface can be argued to be due to the composition of the metal rather than a result of storage environment, holding time or number of reprocessing cycles. The forceps in our study contained 12—14% chromium which is comparable to the surgical scissors examined by Bundgaard *et al*. containing 12.5% chromium [20]. Pitting, embeddings, teeth marks and scratches were equally observed on forceps across storage environment, holding time and number of reprocessing cycles. The question is whether these congenital damages in the metal impose a risk to patient safety in terms of transferring biocontamination from one patient to another.

In our study we observed that the amount of corrosion increased by number of reprocessing cycles. However, we do not know whether corrosion residues from the instruments are transferred to the patient during surgery. Furthermore, we do not know whether (or which amount of) corrosion left inside the patient during surgery poses a health risk for the patient. Two recent studies showed a likely causality between the occurrence of corrosion on bone lengthening nails and serious adverse events such as pain, osteolysis, periosteal reaction and cortical hypertrophy [21,22]. Knowledge in this subject area is sparse and research is strongly warranted.

The main finding of this study challenges the recommendation of preferring a humid storage environment before reprocessing compared with a dry storage environment, as we found no evidence that a humid storage environment increases patient safety or instrument durability. Thus, the cleanliness and durability of the instruments seems not to be affected by storage environment but instead by number of treatment cycles.

Acknowledgements

We owe great gratitude to the staff in the Sterile Centre for their meticulous reprocessing processes of the instruments in the test period. Furthermore, we owe gratitude to the staff in the Department of Clinical Biochemistry for providing human blood.

Conflict of interest statement
None declared.

Funding sources
The study was funded by Health Innovation Fund, North Denmark Region, Denmark.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2022.01.012.

References

[1] Proper maintenance of instruments. 10th edition. Instrument Preparation Working Group; 2012.
[2] Statens Serum Institut. Nationale Infektionshygiejniske Retningslinje for genbehandling af steriliserbart medicinsk udstyr. [National infection hygienic recommendations for reprocessing of sterilizable medical equipment]. Central Enhed for Infektionshygiejne 2019;1.
[3] ISO 17664:2017 Processing of health care products — information to be provided by the medical device manufacturer for the processing of medical devices. 2nd edition October 2017.
[4] Lipscomb IP, Pinchin H, Collin R, Keevil CW. Effect of drying time, ambient temperature and pre-soaks on prion-infected tissue contamination levels on surgical stainless steel: concerns over prolonged transportation of instruments from theatre to central sterile service departments. J Hosp Infect 2007;65:72—7.
[5] Secker TJ, Pinchin HE, Hervè RC, Keevil CW. Efficacy of humidity retention bags for the reduced adsorption and improved cleaning of tissue proteins including prion-associated amyloid to surgical stainless steel surfaces. Biofouling 2015;31(6):535—41.
[6] Secker TJ, Hervé R, Keevil CW. Adsorption of prion and tissue proteins to surgical stainless steel surfaces and the efficacy of decontamination following dry and wet storage conditions. J Hosp Infect 2011;78:251—5.
[7] Costa DM, Lopes LKO, Tipple AFV, Johani K, Hu H, Deva AK, et al. Evaluation of stainless steel surgical instruments subjected to multiple use/processing. Infect Dis Health 2018;23:3—9.
[8] Spruce L. Back to basics: instrument cleaning. AORN J 2017;105:292—9.
[9] Lopes LKO, Costa DM, Tipple AFV, Watanabe E, Castillo RB, Hu H, et al. Complex design of surgical instruments as barrier for cleaning effectiveness, favouring biofilm formation. J Hosp Infect 2018;100:1—9.
[10] ISO 7153-1: 2016, Surgical instruments — Materials — Part 1: Metals. 2016-10.
[11] EN-ISO 15883-1: 2009, "Washer Disinfectors — Part 1: General Requirements, Terms and Definitions and Tests", Annex C, Test Methods for the Detection and Assessment of Residual Proteinaceous Contamination, C .2 'OPA method for the assessment of residual proteinaceous contamination'.
[12] DS/EN ISO 10289, 2001 Edition. Methods for corrosion testing of metallic and other inorganic coatings on metallic substrates — Rating of test specimens and manufactured articles subjected to corrosion tests. May 4, 2001.
[13] Hilsberg P. Restprotein tester. [Protein residue tester] Norsk forening for sterilforsyning Årsmøde 13.- 14. marts 2014. Available at: http://sterilforsyning.no/files/torsdag-1100-restprotein-tester-pia-hilsberg.pdf [August, 2020].
[14] Aasim SA, Mellor AC, Qualtrough AJE. The effect of pre-soaking and time in the ultrasonic cleaner on the cleanliness of sterilized endodontic files. Int Endodont J 2006;39:143—9.
[15] Thomas VC, Thurlow LR, Boyle D, et al. Regulation of autolysis-dependent extracellular DNA release by Enterococcus faecalis extracellular proteases influences biofilm development. J Bacteriol 2008;190:5690—8.
[16] Percin D, Sav H, Hormet-Oz HT, Karauz M. The relationship between holding time and the bacterial load on surgical instruments. Indian J Surg 2015;77:16—8.
[17] Mohite ST, Mahesh Reddy S, Kshirsagar YK, Kabra M, Nagur B, Biradar S. Effect of holding time on the bacterial load of surgical instruments. J Evol Med Dent Sci 2012;5:763—5.
[18] Roberts CG. The role of biofilms in reprocessing medical devices. Am J Infect Control 2013;41:77—80. https://doi.org/10.1016/j.ajic.2012.12.008.
[19] REDA, Technical and Regulatory Data Sheet, Rev.01/16.07.2020TB.

[20] Bundgaard K, Sørensen EE, Ripadal K, Christensen A-E, Schønheyder H-C. Challenging the six-hour recommendation for reprocessing sterizable medical equipment. J Hosp Infect 2019;101:13—9.
[21] Jellesen MS, Lomholt TN, Hansen RQ, Mathiesen T, Gundlach C, Kold S, et al. The STRYDE limb lengthening nail is susceptible to mechanically assisted crevice corrosion: an analysis of 23 retrieved implants. Acta Orthop 2021;92:621—7.
[22] Rölfing JD, Kold S, Nygaard T, Mikuzis M, Brix M, Faergemann C, et al. Pain, osteolysis, and periosteal reaction are associated with the STRYDE limb lengthening nail: a nationwide cross-sectional study. Acta Orthop 2021;92(4):479—84.