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Challenging the six-hour recommendation for reprocessing sterilizable medical equipment

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SUMMARY

Background: At present, reprocessing of sterilizable medical equipment is recommended to be initiated within 6 h after completion of surgery, to ensure that the quality of the instruments does not deteriorate. A literature search showed a lack of evidence for consequences that may occur if medical personnel deviate from the standard 6 h sterilization protocol.

Aim: To evaluate the 6 h recommendation for reprocessing sterilizable medical equipment by determining whether residual protein increased proportional to holding time before reprocessing was initiated, and likewise whether an increase in corrosion was present on surgical scissors proportional to holding time.

Methods: Residual protein was identified on surgical instruments contaminated with human blood after different holding times and before washes using the *o*-phthaldialdehyde (OPA) method. Corrosion was identified on surgical scissors contaminated with human blood after different holding times and after reprocessing using light stereomicroscopy and scanning electron microscopy.

Findings: Protein residues ranged between 14.0 and 51.9 μg and thus below the accepted threshold of 100 μg per instrument surface. Corrosion corresponding to 0.05% of the surface was identified on 22 of 30 scissors. Pitting corrosion was seen on four of 30 scissors.

Conclusion: No association was identified between residual protein and holding time, nor between incidence of corrosion and holding time. The study thereby challenges the relevance of upholding the recommendation of a maximum wait of 6 h prior to reprocessing. The findings will potentially have an impact on the organization of reprocessing of surgical instruments in Denmark and internationally.

Introduction

At present, the reprocessing of sterilizable medical equipment is recommended to commence no later than 6 h after

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completion of surgery, according to national and international guidelines for infection control in the healthcare sector [1–3]. The concern is that a longer holding time may result in deterioration of the instruments, i.e. the instruments may not become clean using standard protocols for reprocessing and may consequently be more susceptible to corrosion. The recommendations for reprocessing of sterilizable medical equipment are described using the term 'should', and medical personnel are advised that reprocessing must be initiated 'as soon as possible'. However, a literature search in Embase, Web of Science, and PubMed revealed a lack of evidence regarding the potential consequences for infection control and risk of corrosion if there is a deviation from the standard 6 h reprocessing window.

The most recent recommendations from a working group representing manufacturers of instruments, disinfectants, cleaning and care agents, washer-disinfectors, and sterilizers states:

> Because of the corrosion risk and the cleaning factors, long intervals between instrument use and reprocessing (e.g. overnight or over the weekend) should be avoided, irrespective of the disposal methods used (i.e. wet or dry). Field experience has shown that in the case of dry disposal, intervals of up to 6 hours pose no problem ([1], p. 30).

This recommendation is found in the 10th edition, from 2012. Since publication of the first edition in 1979, editions have been widely disseminated in 19 languages, reflecting the working group's international relevance. Its recommendations build on guidelines, procedural descriptions and consensus about 'best practices' [1]. Additionally the ISO 17664:2017 standard describes 'The time between medical device use, the initial treatment and/or the next step of the process' to be information that the medical device manufacturer shall provide, where applicable ([3] p. 7).

Research-based knowledge indicates that there is an increase in residual protein, bacterial load, and prion amyloid proportional to holding time before reprocessing is initiated [4–7]. However, the work by Lipscomb *et al.* demonstrated that ' … all pre-soaks significantly reduce (by up to 96%) the prion-infected tissue contamination, and that controlling the temperature while in transit between theatres and cleaning facilities may allow an increase in time before high protein adsorption levels occur' [4]. No research to date has tested the association between holding times exceeding six hours and the cleanliness of the instruments after reprocessing. Additionally, based on reported incidents associated with the unsuccessful decontamination of reusable surgical instruments, the risk of cross-transmission of infection and exposure appears to be very low [8]. These results call for further studies challenging the recommendation of a maximum wait of 6 h before reprocessing. This study therefore set out to evaluate the 6 h recommendation for reprocessing sterilizable medical equipment. We investigated whether an increase in residual protein content is proportional to holding time before reprocessing is initiated, and whether an increase in corrosion is present on surgical scissors proportional to holding time before reprocessing is initiated.

Methods

Simple instruments, such as scissors and knife shafts, and more complex instruments with cavities, such as puncture cannulae, were tested. Defibrinated human blood was donated by consenting, voluntary, unpaid, and anonymous blood donors in accordance with Danish rules [9]. The instruments were contaminated with the blood and then left to dry for 0, 3, 6, 9, 12, 24, and 36 h at room temperature before washing (i.e. dry storage). A sterile cotton swab was soaked in undiluted blood and used to lubricate all surfaces of knife shafts and scissors, and puncture cannulae were flushed with blood. The instruments were washed in the washer-disinfector using the standard protocol for the Sterile Centre at Aalborg University Hospital, Denmark, Appendix 1. After washing, but before disinfection, the instruments were examined for protein residue using the *o*-phthaldialdehyde (OPA) method (Annex C, C. 2 to ISO 15883-1: 2006 (E)).

OPA analysis

The OPA analysis was based on EN-ISO 15883-1: 2009 [10]. For elution the puncture cannulae were placed in sterile 15 mL tubes with 5 mL 1% sodium dodecyl sulphate (SDS), and rinsed using a 5 mL syringe by filling and emptying five times. Items were left in an orbital shaker (200 rpm) at room temperature for 30 min, after which they were rinsed additionally five times. Finally, the tubes were exposed to vortexing for 5 s, prior to transfer of 100 μL per well, within a microplate.

Scissors were eluted with 10 mL 1% SDS in stomacher bags. Scissors were rubbed for 30 s and placed in an orbital shaker (200 rpm) at room temperature for 15 min. The items were turned and again placed in the orbital shaker for a further 15 min. The scissors were again rubbed for 30 s. The eluate was transferred to 15 mL tubes, exposed to vortexing for 5 s prior to transfer of 100 μL per well, within a microplate.

Knife shafts were eluted with 10 mL 1% SDS in stomacher bags. The bags were placed in de-gassed ultrasonic baths where they were sonicated at 40 kHz for 5 min at room temperature. Then the items were left for 20 min, after which sonication was repeated. The eluate was transferred to 15 mL tubes, exposed to vortexing for 5 s prior to transfer of 100 μL per well.

In Denmark the consensus acceptable level for surface protein residues is a maximum of 100 μg/instrument [11].

Corrosion analysis

Corrosion resistance was tested using two qualities of surgical scissors, in order to include metals of different composition. Analyses showed that one quality of scissors had a chromium content of 16%, and the other 12.5%. Fifteen scissors of each type were lubricated with blood on all sides using a sterile cotton swab and left to dry for 6, 12, and 24 h (i.e. dry storage), following which, they were washed, disinfected, and autoclaved. After washing and disinfection, the scissors were inspected for visible signs of corrosion before being autoclaved. The process from contamination to end autoclaving was repeated in the same way 50 times. Pairs of scissors of each quality subjected to each of the three holding times were tested for corrosion after 25, 35, and 45 reprocessing cycles, respectively. The remaining two scissors of each quality and holding time were retrieved after 50 reprocessing cycles. The individual scissors had the same holding time before reprocessing throughout the test period. The scissors were examined and evaluated using light stereomicroscopy and

Table I
Corrosion adjustment scale [12]

Degree of corrosion	Area (%)
Ri 0	0
Ri 1	0.05
Ri 2	0.5
Ri 3	1
Ri 4	8
Ri 5	40–50

scanning electron microscopy (SEM). The degree of corrosion was assessed according to the ISO 4628-3 standard [12]. The number of pictures defines the degree of corrosion from Ri 0 to Ri 5 where Ri 1, for example, corresponded to 0.05% of the instrument surface. Corrosion >50% corresponded to Ri 5 (see Table I). The OPA protein assay, stereomicroscopy and SEM analyses were conducted by the Danish Technological Institute, Aarhus, Denmark.

Data analysis

The aim was to investigate whether there was an association between holding time and the amount of protein residue for the three instrument types separately using linear regression. During the analysis, the protein residues were converted into micrograms on the basis of the linear function of the standard series. The equation used to determine linearity for puncture cannulae was $y = 1.494^*x + 8.928$ ($R^2 = 0.999$). For knife shafts $y = 1.589^*x + 7.168$ ($R^2 = 0.998$) and for the scissors $y = 1.525^*x + 7.749$ ($R^2 = 0.998$).

To investigate whether there was a difference in the distribution of corrosion with respect to the number of reprocessing cycles completed for the two qualities of scissors using Fisher's exact test for each type separately, a spine plot was included for visualization of the proportion of scissors with and without corrosion in terms of the proportion of scissors within each number of reprocessing cycles. To compare the same number of observations per number of reprocessing cycles, the average in cases of repeated observations was recorded, as the variations were expected to be minimal.

This was a small-scale study, hence no power calculation was used to determine sample size.

Results

Protein residue

Table II shows protein residues identified on puncture cannulae, scissors, and knife shafts. The negative controls (instruments that were not contaminated but washed) had the same amount of protein residue as the instruments with holding times prior to washing. Regardless of holding time and instrument type, all protein residues were below the consensus-accepted threshold of 100 μg per instrument surface, with the lowest value at 14.0 μg and the highest value at 51.9 μg. Only three out of 42 values were >50 μg; the remaining 39 values were ≤40 μg. The room temperature in the Sterile Centre during the trial fluctuated from 22.1 to 25.7°C, with the highest temperatures in the late afternoon and during the night.

Table II
Protein residues

Treatment/ holding time (h)	Protein μg/puncture cannulae	Protein μg/scissors	Protein μg/knife shafts
Positive control[a]	>500 (794)[b]	>1000 (2720)[b]	>1000 (2200)[b]
	>500 (1010)[b]	>1000 (2730)[b]	>1000 (2290)[b]
Negative control[c]	12.7	41.4	36.6
	16.0	43.5	37.3
0 h	14.3	39.2	35.0
	14.3	51.9	32.6
3 h	15.0	35.2	35.2
	14.8	35.0	33.4
6 h	16.2	36.3	33.1
	18.5	50.4	33.0
9 h	20.4	40.0	35.5
	25.9	38.2	35.9
12 h	14.0	35.1	33.5
	15.6	34.6	33.6
24 h	15.3	33.7	33.0
	13.8	37.7	32.4
36 h	50.9	35.4	31.7
	14.3	34.5	31.0

[a] Positive controls: instruments that were contaminated but not washed.
[b] The signals for positive controls were out of range for the standard series. The values in the parentheses were found by extrapolation of the linear function for the standard series.
[c] Negative controls: instruments that were not contaminated but washed.

Humidity ranged from 26.4% to 42.4%. However, the fluctuations in temperature and humidity are not considered relevant to the results.

No correlation between holding time and the amount of protein residue was identified for the puncture cannulae. A non-significant slope of −0.37 ($P = 0.09$, 95% CI: 0.07, 0.81) was identified, and $R^2 = 0.216$. The amount of protein residue on the contaminated puncture cannulae varied from 14.0 to 50.9 μg. One of the lowest and the highest values (14.3, 50.9 μg) were obtained from the samples with a holding time of 36 h. The 6 h values of 16.2 and 18.5 μg were higher than the 12 h values of 14.0 and 15.6 μg.

Likewise, there was no correlation between holding time and the amount of protein residue remaining on the scissors. The observed slope was −0.21 ($P = 0.11$, 95% CI: 0.47, 0.06), and $R^2 = 0.196$. Protein residue values for the scissors ranged from 33.7 to 51.9 μg. The two highest values were identified on the scissors with holding times of 0 and 6 h, and 51.9 and 50.3 μg, respectively. A weak correlation was found between holding times and protein residue for the knife shafts, with a slope of −0.08 ($P = 0.01$, 95% CI: −0.13, −0.02), and $R^2 = 0.431$. The protein residue on the knife shafts ranged from 31.0 to 35.9 μg.

Corrosion

Stereomicroscopy showed surface areas with corrosion of the degree Ri 1 (Tables I and III). The corroded areas were identified as those with red-coloured deposits. In addition, lighter discoloration was observed on the scissors; we

Table III
Corrosion data

Cycles	No. of scissors with no corrosion	No. of scissors with corrosion	Total	*P*-value[a]
Scissor 16% chromium				
0	1	0	1	
25	2	1	3	
35	3	0	3	
45	0	3	3	
50	0	6	6	0.05
Scissor 12.5% chromium				
0	1	0	1	
25	1	2	3	
35	1	2	3	
45	1	2	3	
50	0	6	6	1.00

[a] Fisher's exact.

considered this to be caused by detergents and the drying process. The scissors were examined in two areas, as illustrated in Figure 1. The analyses were made only for Area 2, because corrosion grade Ri 1 was observed for both types of scissors for all holding times and for any number of reprocessing cycles in Area 1.

A comparison of the two qualities of scissors showed that the surface structure of scissors with 12.5% chromium was not entirely as smooth as the surface of scissors with 16% chromium. The scissors with 12.5% chromium also appeared to have small silicon embeddings (3 μm in diameter) and were therefore 'born' with small impurities in the surface within which pitting can develop. Examples of silicon embedding and pitting corrosion are illustrated in Figure 2. A higher incidence of corrosion was identified on scissors with 12.5% chromium, where 12 out of 15 scissors were affected, compared to 10 out of 15 scissors with 16% chromium.

Figure 3 illustrates a spine plot for the proportion of scissors with and without corrosion in relation to holding time. In the plot, holding times are illustrated by a colour code and the occurrence of corrosion by 0 and 1, where 0 is no corrosion and 1 is the corrosion degree Ri 1. Similarly, Figure 4 illustrates the proportion of scissors with and without corrosion in relation to

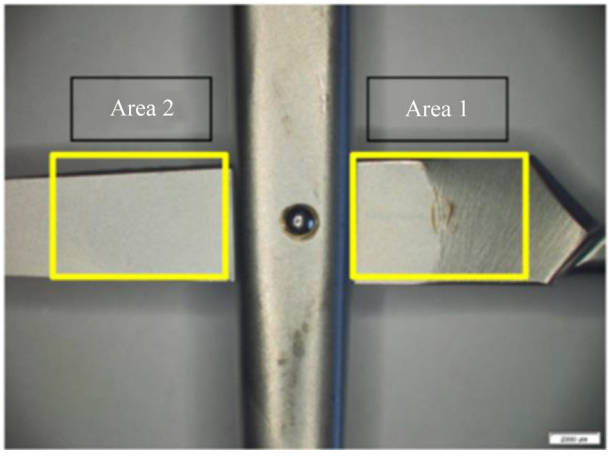

Figure 1. Scissors with markings of Area 1 and Area 2.

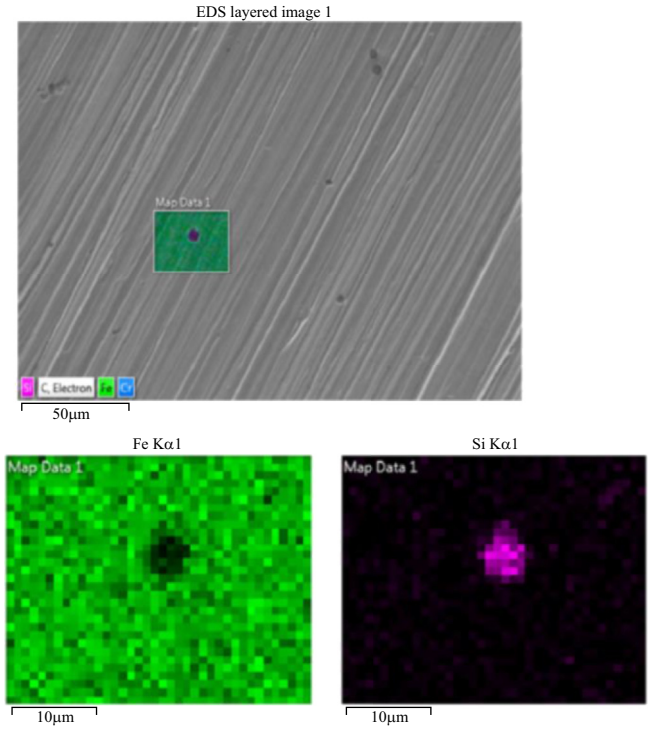

Figure 2. Example of silicon embedding and pitting corrosion.

the number of reprocessing cycles they have been through. The differences in occurrence of corrosion are shown by the size of the coloured areas in Figures 3 and 4.

The light stereomicroscopy showed a weak tendency (no clear signs) toward less corrosive activity on scissors with 16% chromium and holding times of 6 or 12 h, compared to scissors with a holding time of 24 h. There was no clear tendency for the scissors with 12.5% chromium, where the same degree of corrosion was observed on the scissors with holding times of 6, 12, and 24 h. Pitting, indicating severe corrosion attack, was observed in Area 2 on four scissors with 12.5% chromium. These were the scissors with holding times of 12 h and 35 reprocessing cycles, 12 h and 50 reprocessing cycles, and two scissors with holding times of 24 h and 50 reprocessing cycles. It is possible that this pitting corrosion had already begun at inclusion and was caused by the quality of the scissors, not the holding time before reprocessing.

Discussion

Substantiated by recommendations from the Instrument Preparation Working Group and the Medical Devices Agency, Danish guidelines for the healthcare sector strongly recommend that reprocessing of sterilizable medical equipment commence no later than 6 h after the completion of surgery ([2] p. 32) [13,14]. However, the present study questions whether a longer holding time results in increased protein residue contamination and a heightened risk of corrosion. Lipscomb *et al.* found that at 22°C all adsorption of protein to the surface of an instrument will have occurred after ~40 min; furthermore, that at higher temperatures the speed of adsorption will increase [4].

Secker *et al.* showed that different types of storage for surgical instruments prior to reprocessing could reduce the

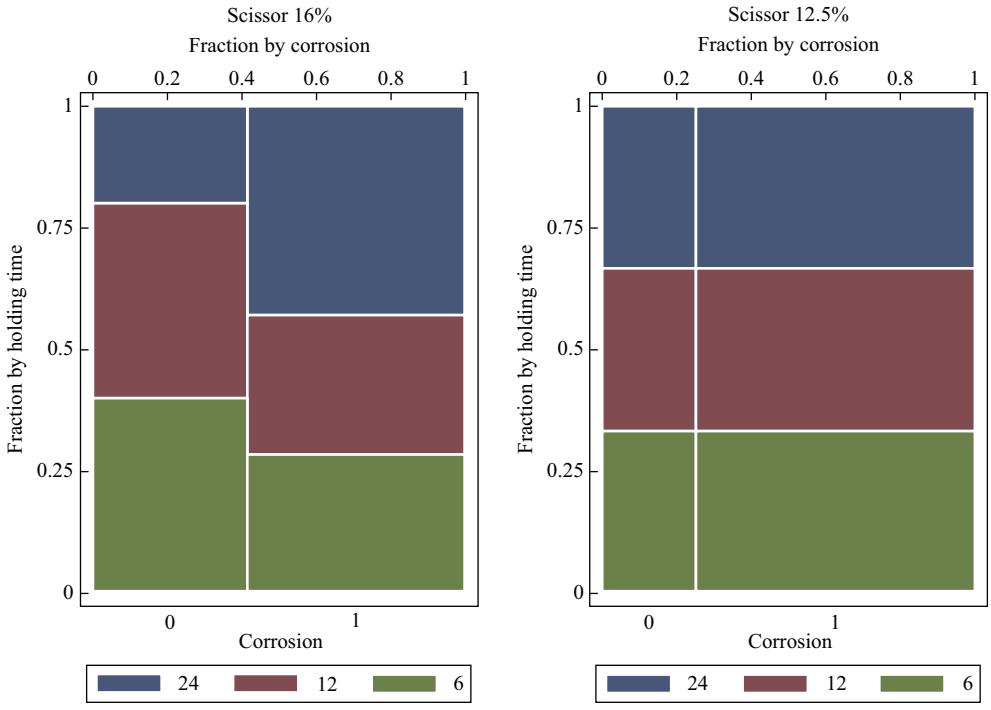

Figure 3. Scissors with and without corrosion in relation to holding time.

adsorption of proteins to surgical steel surfaces [5]. They observed an increase in biological contamination proportional to increased drying times whether preserved dry and uncovered or dry and covered. By contrast, independent of the drying time, they observed a minimal increase in contamination if the steel was stored in a humid environment. According to these studies, the amount of protein adsorbed to the instrument reaches a maximum after 40 min. Even with the use of enzymatic softeners and environmental and temperature control during transportation, full adsorption will have already occurred when the instruments reach the cleaning facilities.

Hence, these studies do not provide evidence that can support the recommendation of a maximum wait of 6 h before reprocessing. The objective of the present study was to imitate current reprocessing practice at the Sterile Centre at Aalborg University Hospital and therefore only instruments stored dry and uncovered were tested. Thus, this study does not clarify whether sterile services should consider using commercially

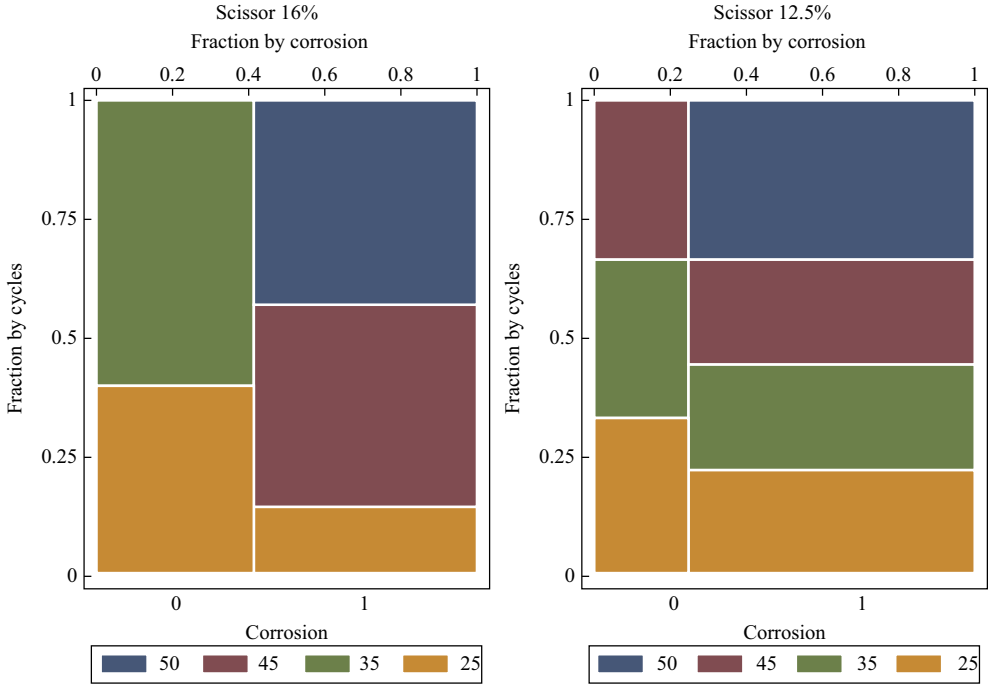

Figure 4. Scissors with and without corrosion in relation to the number of reprocessing cycles.

available wetting agents or processes to maintain moisture and improve cleanability as suggested by Lipscomb *et al.* and Secker *et al.* [4,5]. However, the present study provides evidence for no association between the presence of protein residue on three different types of surgical instruments and the holding time before reprocessing was initiated. Thus, the cleanliness of instruments after dry and uncovered storage seems to be independent of holding time before reprocessing. Furthermore, the study demonstrates that instruments may have holding times up to 36 h before reprocessing is initiated without exceeding the accepted upper limit of protein residue of 100 μg.

The literature search revealed two recent experimental studies addressing augmented bacterial growth on instruments not reprocessed within the recommended 6 h period [6,7]. In both studies, the instruments were contaminated with three common pathogenic bacteria with a quantification of bacterial growth at room temperature at various time-points. A notable increase in the numbers of colony-forming units per square centimetre was reported for a 12 h holding time, compared with a 6 h period. It is not surprising that bacterial growth occurs over time on unwashed contaminated instruments. However, these studies do not answer the question of whether there is a difference in cleanliness after the reprocessing of instruments left with bacterial growth for <6 h, compared to those left for >6 h.

Instead of a surrogate ('test soil') we have used defibrinated human blood. The National Committee on Health Research Ethics has endorsed the use of human blood for research purposes conditional on the blood donor's informed consent. Thereby use of a substitute for human blood is avoided. The addition of *Enterococcus faecium* to 'test soil' is not relevant in this context. The human blood used in the study was not pretreated or cleaned, which means it may have been contaminated with both bacteria and medicine residues. This suggests that the cleanliness of instruments may be independent of holding times before reprocessing.

The study revealed distinct differences in the surface structure of the two qualities of scissors. The surfaces of the scissors with 12.5% chromium were not entirely as smooth as the scissors with 16% chromium and had small silicon embeddings (3 μm in diameter) within which pitting corrosion might develop. This finding emphasizes the higher incidence of corrosion identified on the scissors with 12.5% chromium and that pitting corrosion attacks were only observed in the scissors with 12.5% chromium. These findings are in concordance with Rosenberg's and Kaiser *et al.*'s claim that the corrosion resistance of the steel depends on the amounts and composition of its specific components [15,16].

Finally, this study demonstrated no clear signs for either an association between the incidence of corrosion and holding time before reprocessing was initiated, or the number of reprocessing cycles. Unfortunately, no research-based knowledge has been identified that can challenge or support this finding.

Our study has several limitations. The number of test units for both protein residue and corrosion is a restricting factor. In comparison to protein residue, inclusion of more test units could have enhanced the statistical analysis. In relation to corrosion, a higher number of repetitions of the reprocessing cycle could provide additional knowledge. Likewise, only three different instrument types were included in the study; perhaps the inclusion of other instruments, which may be more complex, could have strengthened the study.

In conclusion, this study found no evidence that a longer holding time results in deterioration of reusable instruments. The three different instruments (scissors, knife shafts, and puncture cannulae) tested in this study all become clean using a standard protocol for reprocessing, and their levels of identified residual protein ranged from 14.0 to 51.9 μg, below the accepted threshold of 100 μg. Furthermore, the study revealed no evidence that two different qualities of scissors are more susceptible to corrosion when holding times exceed 6 h. The study clearly challenges the relevance of upholding the recommendation of a maximum wait of 6 h before reprocessing.

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Conflict of interest statement
None declared.

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Appendix 1. Washing procedure details

Manufacturer	Miele
Model	Miele Professional PG 8855
Detergent	Neodisher Mediclean Forte (working solution pH 10.3) Neodisher Mediklar (pH 5.8)
Meets the requirements	DS/EN/ISO 15883
Water quality	Soft water combined with reverse osmotic water During the drying process, the air is passed through a HEPA filter H13
Program	Special vario A0 value >3000 Pre-rinse: Cold water <45°C Thermic disinfection 93°C for 5 minutes Drying with air up to 110 °C for 20–25 minutes
Loading of instruments	The test instruments have been placed in wired mesh baskets with open box locks The test instruments have been randomly loaded in washing racks together with instruments used for surgery

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