Original article

Brushless cleaning of PTFE test specimens as a surrogate for endoscope channels

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Conflict of interest:
All authors confirm that there is no conflict of interest according to the guidelines of the International Committee of Medical Journal editors (ICMJE).

Citation:
This article is an authorized English translation of the German original publication. Please quote the German original article from the journal Hygiene & Medizin as follows: Rehl J., Lämmer P, Klein N, Hammann HG, Gemein S, Exner M, Gebel J. Bürstenlose Reinigung von PTFE-Prüfkörpern als Surrogat von Endoskopkanälen. Hyg Med 2020; 45(12): D138-D145.

Manuscript data:
Submitted: 08.10.2020
Revised version accepted: 20.11.2019

Summary:

Introduction: The reprocessing of reusable endoscopes requires a pre-cleaning, which up to now has been carried out manually as brush cleaning by a specialist. The unpredictability of the „human factor" leads to the urgent concern to replace this manual cleaning before mechanical disinfection by a mechanical process. Studies have shown that the Comprex® impulse rinsing process is suitable for this purpose.

Methods: Endoscope-channel dummies (PTFE test specimens) with heparinized and protamine-added sheep blood were used as test specimens. These dummies complied with Annex 8 of the Guideline for the Validation of Mechanical Cleaning and Disinfection Processes for the reprocessing of thermolabile endoscopes [1, 2]. In addition, the blood contamination was modified with different protamine concentrations (80–150 µl/9.5 ml blood). These contaminated PTFE test specimens were treated in comparison with the previously used brush cleaning and with the machine/automatic Comprex® impulse rinsing procedure at different settings both alone without and in combination with different cleaning agents. Subsequently, the protein analysis of the potentially remaining residues is carried out using the OPA method.

Results: Brush cleaning in combination with enzymatic detergent falls below the guideline value of 0.8 µg/cm² in protein analysis with the exception of experiments with too viscous blood (150 µl protamine/9.5 ml sheep blood). With the Comprex® impulse rinsing method, even these soils can be removed below the guideline value without adding the enzymatic cleaner. With the addition of the enzymatic cleaner, the Comprex® impulse rinsing procedure achieves values of 0.02 µg/cm², which is far below the current guideline value.

Discussion: This study clearly shows that in the case of PTFE test specimens contaminated with blood, the cleaning success with the automatic Comprex® impulse rinsing method is at least equal to that of brush cleaning. With regard to occupational health and safety requirements and requirements for the validation of the cleaning step in the reprocessing of endoscopes, operators should prefer a mechanical process when selecting the procedures.

Keywords
- endoscope reprocessing
- brush cleaning
- brushless cleaning
- pulse rinsing process

1. Introduction

Endoscopy is an indispensable method for diagnostics and treatment in modern medicine. Due to the reusability of the devices, it is highly necessary to reprocess the endoscope efficiently after use to prevent the transmission of infection. Before reprocessing in the cleaning and disinfection device for flexible endoscopes (RGD-E), manual cleaning stages were carried out, in particular pre-cleaning in the test room and brush cleaning of the channels in the reprocessing room. This meant that the staff had close contact with contaminated endoscopes or cleaning uten-

sils and may also have been exposed to the transmission of pathogens. In addition, the success of the cleaning process cannot be validated without limitation. This depends on the respective worker who carries out the cleaning process. The manual cleaning process is difficult to standardise and validate due to the "human factor", the reprocessing documentation is very complex. An alternative automated mechanical process would therefore be very desirable.

Examinations of contaminated endoscope channel dummies (PTFE test specimens) showed that the Comprex® impulse rinsing process (called the Comprex® process in the following) can be an alternative to brush cleaning [3]. Combined with chemical cleaning and disinfection, microbiological contaminations can even be removed to such an extent that the microbiological detection reaches a level below the detection limit.

1.1 Task

The aim of this work was to compare this mechanical process with conventional manual brush cleaning. The resulting findings are important for the use of the Comprex® process in automatic devices.

1.2 KRINKO-BfArM-recommendations

For cleaning endoscopic channels, the Robert Koch Institute (RKI) and the Federal Institute for Drugs and Medical Devices (BfArM) recommend, among other things, the two cleaning stages below [4]:

- Pre-cleaning directly after the test by flushing
- Brush cleaning as part of the reprocessing directly before mechanical reprocessing

According to this recommendation, when brush cleaning, a disinfected brush which fits each channel must be used. However, it is complicated to ensure that the contaminated brushes are also reprocessed safely. This suggests the use of disposable brushes, but means that for each channel, a separate new brush must be used. This is the only possible way to safely avoid transmissions from contaminated channels.

1.3 Problems

In practice, brushes are normally used several times. Both the cleaning and disinfection of brushes used several times and the cleaning of the channels with the brushes are subject to individual variability. In addition, the channels can also be damaged during incorrect brushing and, under certain circumstances, these damaged channels may be reused due to insufficient monitoring possibilities. Manual brushing depends greatly on the person carrying out the task. The staff could become tired during the work. A loss in concentration results in the risk of contaminating oneself and the environment, as well as cleaning the channels insufficiently.

1.4 Efficiency of the cleaning

In the DGKH, DEGEA, DGSV, DGVS and AKI guideline for validating mechanical cleaning and disinfection processes for reprocessing thermolabile endoscopes, specific test specimen models were described in appendix 8 to present the cleaning efficiency. These test specimen models consist of 2-m-long PTFE hoses (PTFE test specimens) which are filled with sheep's blood [1, 2].

According to the standardised treatment, the residual proteins are eluted with SDS solution and the protein is detected using the modified *ortho*-phthaldialdehyde (=OPA) method.

Without treatment, the PTFE test specimens should show recovery rates of between 70% and significantly lower than 100% of the previous protein load.

The Wehrl [5] findings of a field study published in 2016, in a communication of the task group of DGKH, DEGEA, DGSV, DGVS and AKI, where endoscope and cleaning as well as disinfecting agent manufacturers were involved, about updating the acceptance criteria for guideline test specimens after the cleaning stage, led to the following [6]:

- the test specimens must be visually clean
- the reference value of ≤100 µg protein/test specimen (corresponds to ≤ 0,8 μg protein/cm²) must be adhered to

2. 2. Material and methode

2.1 Cleaning stand

After the examinations of an existing cleaning stand 1, the findings from a research plan [3] influenced a new, automated cleaning stand 2. With this automated cleaning stand 2, one or two PTFE test specimens could be integrated and cleaned using various processes (fig. 1 and 2).

2.2 Materials

- Sheep's blood in Na-heparin (10 I.U./ml), sterile, Fiebig-Nährstofftechnik
- Protamine ME 1000 I.E./ml; MEDA Pharma GmbH & Co.KG
- Cleaning agent 1: neodisher MultiZym enzymatic cleaning agent; Dr. Weigert GmbH & Co.KG
  According to information from the manufacturer, for manual cleaning with a high degree of contamination, 2.5 – 30 ml/l are prescribed for 15 – 50 °C and a working time of 2 – 10 min.
- Cleaning agent 2: Sekusept aktiv; disinfection cleaning agent, Ecolab Deutschland GmbH
  According to information from the manufacturer, the product can be used for low and high degrees of contamination with a concentration of 2% and a working time of 5 min.
- Cleaning brushes:
  BW-422T Ø 2 to 4.2 mm, 2200 mm length; Olympus Deutschland GmbH
  BW-201T Aset Ø 2 to 4.2 mm, 2200 mm length; Olympus Deutschland GmbH

2.3 Test contamination in PTFE test specimens

2-m-long, new PTFE hoses with an internal diameter of 2 mm and an external diameter of 3 mm act as test specimens. The internal area is 12,564 mm² or 125.64 cm². The volume is calculated as 6,280 mm³ or 6.28 ml. These PTFE hoses correspond to the test specimens stated in appendix 8 of the DGKH guideline [1, 2].

As test contamination, heparinised sheep's blood was produced following DIN EN ISO 15883-4 [7] and the DGKH guideline [1, 2]. Depending on the test series, the ratio of protamine to sheep's blood varied between 80 μl and 150 μl protamine/9.5 ml sheep's blood, which meant that the cleaning success with the automated Comprex rinsing process at least corresponding with the manual brush cleaning in the case of PTFE test specimens contaminated with blood.

The treated sheep's blood was first drawn into a 10 ml disposable syringe without air bubbles and then injected into the PTFE test specimen. After an

incubation period of 30 seconds, two 10 ml rounds of air were pressed into the PTFE test specimen using a disposable syringe. The PTFE test specimens were kept still in a horizontal position at room temperature for one hour (fig. 3) before they were cleaned after a storage period of maximum two hours after being checked for penetrability.

2.4 Cleaning the contaminated PTFE test specimens with brushes

To determine the influence of brush cleaning, PTFE test specimens were prepared with different protamine quantities (80 $\mu g/cm^2$ – 150 $\mu g/cm^2$).

After a storage period of maximum 2 hours, the PTFE test specimens were placed in a cleaning basin with fresh water and cleaned using special brushes. Flexible cleaning brushes of 2 m in length were used, with diameters of 2 mm and 4.2 mm (type BW-422T und type BW-201T), as well as a special plastic cleaning basin with a preformed recess in which to place the endoscope. The PTFE test specimens were subjected to as many brush penetrations as it took until a clear, no longer bloody, flow of liquid was visible at the end of the PTFE test specimen lumen. Then, the test specimens were flushed with 50 ml fresh water. The protein analysis of the treated test specimens then took place in accordance with appendix 8 of the DGKH guideline [2].

The influence of cleaning agents was assessed using two cleaning agents. For this, the cleaning basin was filled with 0.5% neodisher MultiZym, or 2% Sekusept aktiv (media temperature 25 °C), the PTFE test specimens were added and, after 5 mins working time, the brush cleaning described above was carried out. Then, the PTFE test specimens were flushed with 50 ml fresh water and sent for protein analysis.

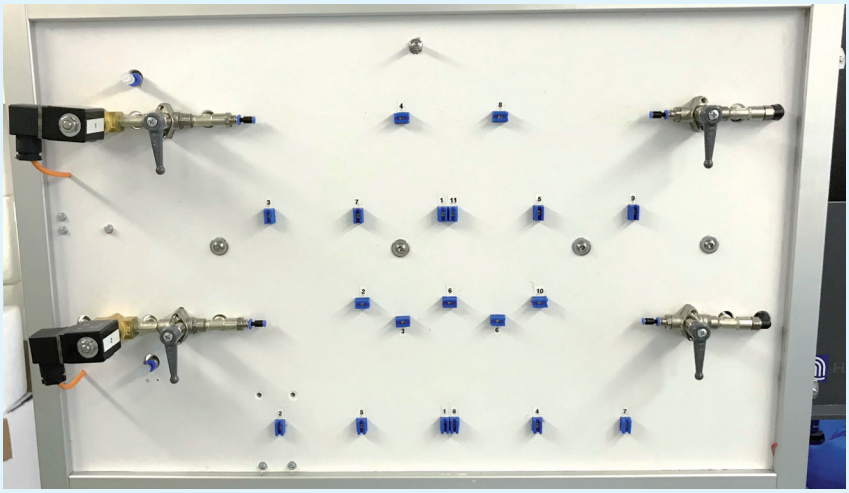

Fig. 1: Cleaning stand 2 with connections for two PTFE test specimens

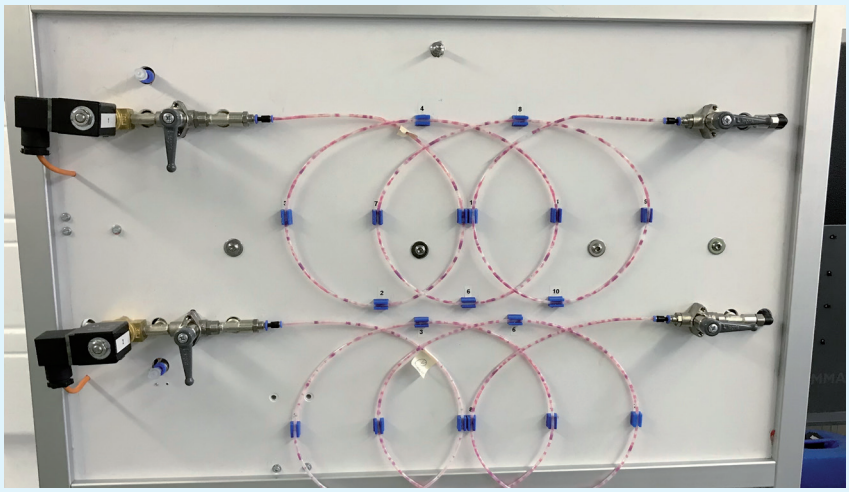

Fig. 2: Cleaning stand 2 with two PTFE test specimens before treatment

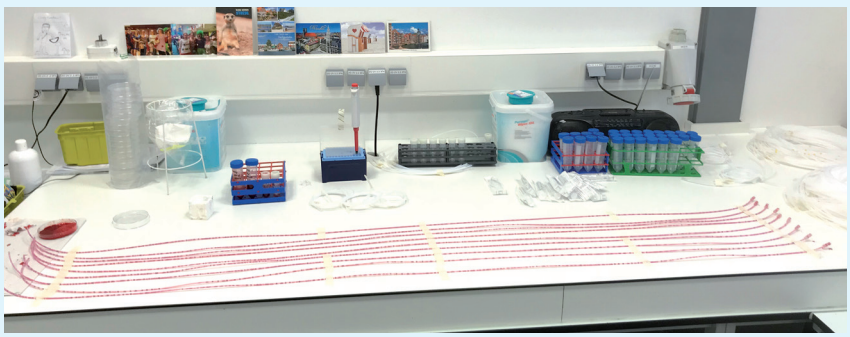

Fig. 3: Horizontally positioned PTFE test specimens after inoculation during the one-hour rest phase

2.5 Cleaning the contaminated PTFE test specimens with impulse rinsing

The PTFE test specimens were fixed into the special devices on the cleaning stands and then treated using the impulse rinsing process (fig. 4). Here, constant water pressure (0.5 bar on cleaning stand 1 and 0.2 bar on cleaning stand 2) and varying air impulse rinse pressure was applied. The number of impulses was 90 or 120. Then, the PTFE test specimens were subjected to protein analysis in accordance with appendix 8 of the guideline [2]. As a control, PTFE test specimens were treated without compressed air impulses only following the constant water pressure described above.

Tests with two cleaning agents with different modes of action were to show whether the physical cleaning effect could be strengthened. To do this, after a determined number of impulses, the PTFE test specimens were filled with different concentrations of the cleaning agents neodisher MultiZym or Sekusept aktiv, and after 5 mins working time, treated with different numbers of impulses. The protein analysis was then carried out in accordance with appendix 8 of the guideline [2].

3. 3. Results

3.1 Cleaning the contaminated PTFE test specimens with brushes

First, an attempt was made to remove the test contaminations from the PTFE test specimens with various ratios of protamine to sheep's blood by brushing.

Here, it was learned that with ratios of more than 130 µl protamine to 9.5 ml heparinised sheep's blood, the brush cleaning was extremely laborious to almost impossible. Due to the high level of viscosity and the resulting high level of resistance, it was barely possible to convey the brush completely through the 2-m-long PTFE test specimen. Sometimes the brushes even broke or cracked during the cleaning tests. For this reason, the 150 µl prescribed by the guideline was not used for the brush cleaning; instead, 120 µl or less protamine/9.5 ml sheep's blood was used.

The cleaning performance was assessed by the determination of residual protein content in the PTFE test specimens after treatment using a modified OPA method and a photometric extinction measurement [1, 2]. The residual protein content, volume and internal area of the PTFE test specimens allowed the calculation of the specific residual protein mass (RP) per cm^2.

The relationship between the different ratios of protamine/sheep's blood and the cleaning performance of both brushes is shown in table 1. This table shows that as the ratio of protamine/sheep's blood is increased, the cleaning performance is reduced. With the brush type BW-422T, the reference value required by the guideline [1, 6] of 0.8 µl/cm^2 can be achieved up to the ratio of 120 µl protamine/9.5 ml sheep's blood and thus the cleaning performance required. With brush type BW-201T, the cleaning performance is only sufficient with lower ratios.

The results in table 2 confirm the assumption that the use of cleaning agents improves the result. The cleaning agent containing enzymes, neodisher MultiZym 0.5%, shows better cleaning effects when compared to the disinfectant Sekusept aktiv 2% for both contaminations investigated.

3.2 Flushing the contaminated PTFE test specimens with fresh water

In a preliminary investigation in cleaning stand 1, PTFE test specimens (test contamination in the ratio of 150 µl protamine/9.5 ml sheep's blood) were flushed with fresh water (0.5 bar for 5 min without additional compressed air impulse). After this treatment, significant quantities of test contamination were still visually noticeable in the PTFE test specimen (fig. 5).

3.3 Cleaning the contaminated PTFE test specimens in the Comprex® process

Orientating tests using both available cleaning stands showed that, unlike manual brush cleaning, the automated Comprex® process can also remove test contaminations in the ratio of 150 µl protamine/9.5 ml sheep's blood from the PTFE test specimens. Further tests were carried out with 90 or 120 impulses using air impulse pressure of 2.0 bar or 3.5 bar. Table 3 shows the findings from non-automated cleaning stand 1.

Table 3 shows that greater pressure and a higher number of impulses improves the cleaning performance, but is not sufficient in every case to achieve the reference value of 0.8 µg protamine/cm^2 required by the guideline [1, 6]. The fluctuations and deviations are a result of the manual approach. Further improvement is achieved by automated cleaning stand 2 (see table 4).

Table 4 shows that the increase of air impulse pressure from 2.0 to 3.5 bar improves the cleaning performance in such a way that the threshold value of 0.8 µg protamine/cm^2 is achieved.

3.4 Comprex® process combined with cleaning agents

After the first phase of the Comprex® cleaning process, there was a five-minute soaking phase where the respective detergent was added in the concentrations recommended by the manufacturers. However, in the case of neodisher MultiZym, only in the concentration 0.5%.

Table 5 impressively shows the improved cleaning performance through the use of cleaning agents against the Comprex® process without adding a cleaning agent with the same parameters such as air impulse pressure, total number of impulses and the prescribed test contamination prescribed by the guideline [1] in three ratios of protamine/sheep's blood. Lower ratios of the protamine/blood approach also showed an improved cleaning performance.

The residual protein content (RP) in the tests carried out always remains below the required reference value of 0.8 µg/cm^2 of the internal area when the Comprex® process is used and when cleaning agents are added. Further tests with greater concentrations of the enzymatic cleaning agent (neodisher MultiZym) recommended by the manufacturer showed no improvements of the cleaning results (results not shown).

Fig. 4: PTFE test specimens in cleaning stand 2 after Comprex® cleaning

Table 1: Cleaning performance after brushing with two brush types in fresh water, depending on the ratio of protamine/sheep's blood.

Brush type	Protamine in µg/9.5 ml sheep's blood	Number of PTFE test specimens	Average RP [µg/cm²]	Standard deviation [µg/cm²]
BW-422T	80	6	0,44	0
	120	18	0,65	0,42
	150*	1	3,38	
BW-201T	80	3	0,78	0,30
	120	29	1,16	0,98
	150*	1	2,64	

*The tests with 150 µg protamine/9.5 ml sheep's blood were only carried out once as the specimens were not penetrable.

Table 2: Cleaning performance after brushing with the brush type BW-422T in different cleaning agents, depending on the ratio of protamine/sheep's blood with a working time of 5 min.

Cleaning agent	Protamine in µg/9.5 ml sheep's blood	Number of PTFE test specimens	Average RP [µg/cm²]	Standard deviation [µg/cm²]
neodisher MultiZym 0,5%	80	3	0,03	0
	120	9	0,09	0,06
Sekusept aktiv 2%	80	3	0,18	0
	120	3	0,45	0,03

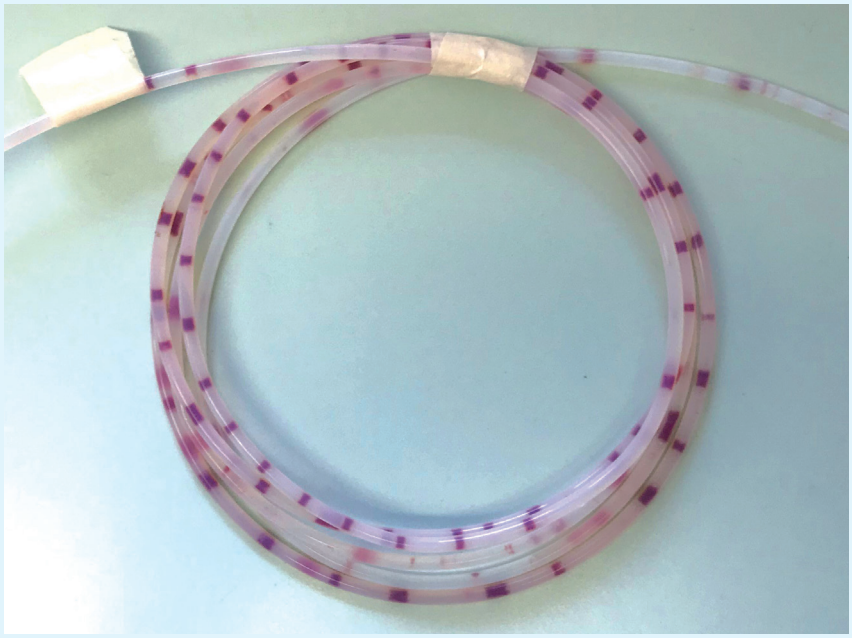

Fig. 5: PTFE test specimen after 5-minute flushing with fresh water (0.5 bar)

3.5 Comparison between the automated Comprex® process and manual brush cleaning

Tables 1 and 2 show that the cleaning result depends on the brush type. However, both brush types are not suitable for stubborn contaminations of 150 µg protamine/9.5 ml sheep's blood. The combination of brush cleaning with the enzymatic cleaning agent had the best cleaning effect, in which the reference value of 0.8 µg/cm² required by the DGKH guideline [1, 6] is safely adhered to. In fact, the value is lower than the reference value by a factor of 10.

Tables 4 and 5 provide information about the findings for automated Comprex® cleaning stand 2. Here, it can be seen that the purely mechanical Comprex® process with 3.5 bar air impulse pressure is already sufficient to achieve the reference value of 0.8 µg/cm². Stubborn contaminations of 150 µg protamine/9.5 ml sheep's blood can also be removed. As with brush cleaning, combining this with cleaning agents gives significantly better results than just cleaning with fresh water. This is why it is appropriate to compare these results.

Depending on the degree of contamination (80 µg to 120 µg protamine/9.5 ml sheep's blood), the average RP values for manual brush cleaning and disinfectant are between 0.18 µg/cm² and 0.45 µg/cm²; for the automated Comprex® process and disinfectant, between 0.26 µg/cm² and 0.38 µg/cm². The use of the enzymatic cleaning agent improves the result for manual brush cleaning to values between 0.03 µg/cm² and 0.09 µg/cm²; for the automated Comprex® process, to values between 0.02 µg/cm² and 0.06 µg/cm².

4. Discussion

For the cleaning of flexible endoscopes, the KINKO-BfArM recommendations [4] differentiate between the pre-cleaning directly after the test in the test room and the brush cleaning of the endoscope channels in the reprocessing room (see table 6).

Figure 5 shows that flushing the channels with water has only a low cleaning performance. As known from other deposits, for example sedimentation in pipe systems [8], the remaining contaminations age with time and become difficult to remove later. Orientating

Table 3: Cleaning performance at cleaning stand 1 with fresh water, depending on the ratio of protamine/sheep's blood at 0.5 water pressure, 2 bar or 3.5 bar air impulse pressure and 90 or 120 impulses.

Air impulse pressure [bar]	Protamine in µg/ 9.5 ml sheep's blood	Number of impulses	Number of PTFE test specimens	Average RP [µg/cm²]	Standard deviation [µg/cm²]
2	80	90	4	0,56	0,04
	120	120	12	1,31	0,66
	150	90	10	1,44	0,76
	150	120	6	0,72	0,31
3,5	80	90	1	0,57	
	120	90	3	0,62	0,07
	120	120	3	1,00	0,66
	150	90	5	1,45	0,42

Table 4: Cleaning performance at automated cleaning stand 2 (fresh water, 2 bar and 3.5 bar air impulse pressure, 90 impulses) depending on the ratio of protamine / sheep's blood.

Air impulse pressure [bar]	Protamine in µg/9.5 ml sheep's blood	Number of PTFE test specimens	Average RP [µg/cm²]	Standard deviation [µg/cm²]
2	80	6	0,51	0,00
	82,5	20	0,93	0,68
	120	40	0,95	0,63
	150	107	1,17	0,59
3,5	82,5	6	0,86	0,30
	120	10	0,67	0,23
	150	21	0,80	0,19

Table 5: Comparison of the cleaning performance at automated cleaning stand 2 (2 bar air impulse pressure, 90 total impulses (where cleaning agents are added 55 impulses before and 35 after the cleaning agent is added), different ratio of protamine/sheep's blood) with enzymatic cleaning agent, disinfectant and without cleaning agent (values from table 4).

Cleaning agent	Protamine in µg/ 9.5 ml sheep's blood	Number of PTFE test specimens	Average RP [µg/cm²]	Standard deviation [µg/cm²]
neodisher MultiZym 0,5 %	82,5	3	0,02	0,03
neodisher MultiZym 0,5 %	120	9	0,06	0,02
neodisher MultiZym 0,5 %	150	27	0,06	0,05
Sekusept aktiv 2 %	82,5	3	0,38	0,18
Sekusept aktiv 2 %	120	9	0,26	0,14
Sekusept aktiv 2 %	150	14	0,48	0,24
No cleaning agent	82,5	20	0,93	0,68
No cleaning agent	120	40	0,95	0,63
No cleaning agent	150	107	1,17	0,59

tests in hoses which are only flushed with water show clear residual contaminations (figure 5) and indicate that high requirements must be set for the cleaning process. The mechanical treatment with a brush in a liquid-filled tub has risks for the cleaning personnel due to any pathogens which may be present. This is why it seems appropriate to combine the cleaning phases "mechanical" and "chemical cleaning", and to carry out this combined cleaning process using machinery. The impulse rinsing in an automated Comprex® cleaning stand – combined with the use of cleaning agents – achieves good cleaning results. Enzymatic cleaning agents have turned out to be especially advantageous, as these generate an effective cleaning performance through the catalytic effect of enzymes. It is highly likely that the Comprex® process, combined with the enzymatic cleaning agents, can be optimised with regard to the Comprex® settings and cleaning agent concentration, to achieve the best possible cleaning performance with the shortest cleaning duration.

Fundamentally, it can be stated that for the validation of a process, preference should be given to the automated/mechanical process over the manual process. Taking into account the fact that brush cleaning can only cover part of the channels, and the brush cleaning is subject to large fluctuations due to the manual approach, the technology presented in this article should be shifted to the focus of risk regulation for those responsible. The insufficient cleaning due to the breaking of the brushes in the case of highly viscous test contaminations in particular shows an inadequate cleaning effect in practice. But this automated process offers many advantages in terms of health and safety at work as well.

In addition, this technology could also be used for sampling reprocessed endoscopes. Sohn et al. [9] demonstrated the superiority of a turbulent fluid flow over pure flushing or a combination of flushing, brushing followed by more flushing (flush-brush-flush). As the process presented here is a pulsed process with water and air blocks, we can expect an even greater cleaning performance and enhancement.

Table 6: "Overview of the various reprocessing types for endoscopes" modified from "Requirements of hygiene when reprocessing medical devices" [4].

	Manual or mechanical investigation	Mechanical
Pre-cleaning	Directly following the test in the test room: wiping the endoscope outer coat and flushing the channels	
Brush cleaning of the endoscope channels	Careful manual cleaning in the reprocessing room (use the disinfected brush that fits each channel!)	
Cleaning flushing	Manually in the reprocessing room	in RDG-E
Disinfection	Insertion with no air bubbles Flushing with disinfectant	in RDG-E
Final flushing	In reprocessing room	in RDG-E
Drying	Manually in the reprocessing room (blown through with compressed air)	in RDG-E

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