Original Article

Comparative assessment of the effects of process parameters as well as of detergents on PTFE channels with regard to automated reprocessing of flexible endoscopes

W. Michels

Corresponding author:
Dr. Winfried Michels
Prüflabor DWM
Kasseler Tor 20
34414 Warburg, Germany

prueflabor-DWM@gmx.de

Conflict of interest:
These studies were sponsored by the firm Schülke & Mayr GmbH but without influencing study conduct, results or evaluation.

Abstract

The cleaning of PTFE tubes or the working channels of flexible endoscopes in washer-disinfectors (EWD), especially in the limit range of the protein elimination currently considered acceptable, has hardly been studied so far. In order to test and evaluate both the cleaning effect of different detergents and different parameters of the cleaning stage, a test setup was created. Using this setup, PTFE hose sections of 10 cm in length, each soiled with 20 µl of reactivated heparinised sheep blood and then conditioned, were subjected to the various cleaning conditions. After cleaning, samples for protein quantification were obtained by cutting the hose sections into 4–5 mm long segments and extracting them with 3 ml 1% sodium dodecyl sulphate solution by vortexing, achieving a recovery rate of 99.8%. All the cleaners tested contained more or less enzymes. The four cleaners with a mildy-alkaline pH and a proportion of anionic surfactants provided results for the residual protein below the detection limit at 3 minutes exposure time and temperatures of 45 and 55°C, respectively. The neutral cleaner with exclusively non-ionic surfactants, on the other hand, led to residual amounts of protein, which amounted to 25 µg per test specimen on average at 35°C and to as high as 86 μg at 55°C, due to the onset of fixation. According to the acceptance criterion of 3 µg/cm² defined by the DGKH, DGSV and AKI guidline group, less than 18.9 µg residual protein would have to be achieved for the hose section. With reference to the type test, the cleaning conditions are suggested by manufacturers of RDG-E as unchangeable. However, the results of these studies show that the use of alternative cleaners and more appropriate cleaning parameters can lead to a significant improvement of the cleaning process and thus an increase in patient safety.

Keywords
- PTFE tube material
- working channels of flexible endoscopes
- cleaning chemistry for AER
- cleaning parameters

Introduction

To date, little research has been carried out on cleaning the PTFE tubes or channels of flexible endoscopes and, in particular, there is little knowledge of the effects of various detergents or of changes made to the process parameters during the cleaning step of automated processes. The processes taking place in the flexible endoscope washer-disinfectors (EWDs) differ in accordance with the manufacturer and distributor. There are also suppliers who by invoking the type test strictly prohibit adaptation of the process parameters to the conditions prevailing at the site of use. That is incomprehensible since making such changes is absolutely normal in the case of the washer-disinfectors (WDs) used to reprocess medical devices and also include thermal disinfection. Nor does standard EN ISO 15883 support such a prohibition. In general it is unclear how the process parameters governing the cleaning steps in EWDs, in particular the conditions determining the use of detergents, were defined.

Currently, the test model specified in the *Guideline for validation of automated cleaning and disinfection processes for reprocessing flexible endoscopes*, compiled by the DGKH, DEGEA, DGSV, DGVS and AKI*, is used to assess the efficacy of

Citation:
Michels W. Comparative assessment of the effects of process parameters as well as of detergents on PTFE channels in terms of automated reprocessing of flexible endoscopes. Zentr Steril 2019; 27 (1): 52–56.

Manuscript data:
Submitted: 21 October 2018
Revised version accepted: 9 January 2019

the cleaning steps in endoscope washer-disinfectors (EWDs) [1, 2]. To that effect, the 200 cm long PTFE (polytetrafluorethylene) tubes with internal diameter of 2 mm, which are used as process challenge devices (PCDs), are contaminated with heparinised and reactivated sheep blood and eluted after cleaning with 1% SDS (sodium dodecyl sulphate) solution by means of rinsing and soaking at intervals. Sampling with the specified method should yield a protein recovery rate of at least 70% for the positive control, i.e. the PCDs with the baseline contamination load [2]. What recovery rate is assured by the described sampling method cannot be inferred from its description, nor is that made clear by the quantitative results of a field study [3]. The PCD has a baseline load of well over 25000 μg protein. For an 80% recovery rate this would mean that well over 5000 μg protein would not be recovered, which would mean a load of well over 40 μg protein/cm². That is precisely the protein-containing deposit coming into direct contact with the PTFE material. Since PTFE adsorbs significant protein amounts [4, 5] it is the removal of exactly these protein amounts that is of pivotal importance for assessment of the cleaning efficacy and is indispensable for assuring a high recovery rate.

Due to that unsatisfactory state a new test model was developed which permits easy verification of the cleaning efficacy of PTFE tubes as well as differentiated assessment of the findings thanks to the high recovery rate assured by the sampling method.

Materials and Methods

To test the cleaning efficacy of PTFE tubes a model consisting of a circulation cleaning system was developed (Figure 1). A 600 ml beaker that was filled with 300 ml of the test cleaning solution served as a liquid reservoir. This was placed on a magnetic stirrer and kept at a set temperature by means of a heating and temperature control facility (C-MAG HS7, Carl Roth, Karlsruhe). A membrane liquid pump LIQUIPORT NF100KT.18S (Article EL62.1, Carl Roth) was used as circulation pump. This pump was used to draw in the cleaning solution by means of a tube suspended in the beaker; the solution was then fed via an adaption point for a 10 cm PTFE tube segment with 2 mm internal diameter (Article 1173.1, Carl Roth), which served as a PCD, and then fed back into the beaker. The tube material used was *Rotilabo* silicone tube with an internal Ø of 6 mm and external Ø of 9 mm (Article 9572.1, Carl Roth). Using that setup, it was possible to set a flow rate of 250-300 ml per minute through the 10 cm PTFE tube segment, used as a PCD, as determined for a similar 2 m long PTFE tube in a commercially available EWD. Before cutting into 10 cm segments the PTFE tube was subjected once to alkaline cleaning in a WD using an instrument programme at 55°C, 10-minute exposure time and the detergent thermoShield Cleaner, Dr. Schumacher, Malsfeld, followed by thermal disinfection at 90°C with 10-minute exposure time.

To maintain the entire system at a constant temperature a bypass consisting of two *Rotilabo* 3-way valves (Article 1018.1, Carl Roth) was devised. During the conditioning period and when changing the tube segment, the solution circulated via this bypass. Because of heat loss from the circulation system the temperature of the liquid reservoir was always set 3°C higher than the nominal cleaning temperature.

The pump had to be stopped for a short period each time the valve settings were changed for integration or replacement of a PTFE tube segment. Following integration of a contaminated tube segment and to start circulation of the detergent solution through the tube segment, the pump was operated at only 1/3 of the volume flow rate and this was then increased within the next few seconds to the marked volume flow rate of 250-300 ml/min.

Heparinised sheep blood (Article 2132005, ACILA GmbH, Mörfelden) whose coagulation was reactivated immediately beforehand by addition of protamine sulphate was used for contamination of the tube segments. To contaminate the tube segments on the inside these were secured to a stand clamp and, using a Hamilton microliter syringe 702 N (Article X034.1, Carl Roth), 20 μl blood was introduced, from each side about half the volume. Using the needle, the blood was then spread on the inside walls.

Following reactivation of the sheep blood that amount of blood was enough to contaminate five tube segments on the inside. Any attempt to increase that number risked causing blockage of the Hamilton syringe, hence it could no longer be used. To ensure that enough time was available for contamination, it was decided to reactivate and use the sheep blood at refrigera-

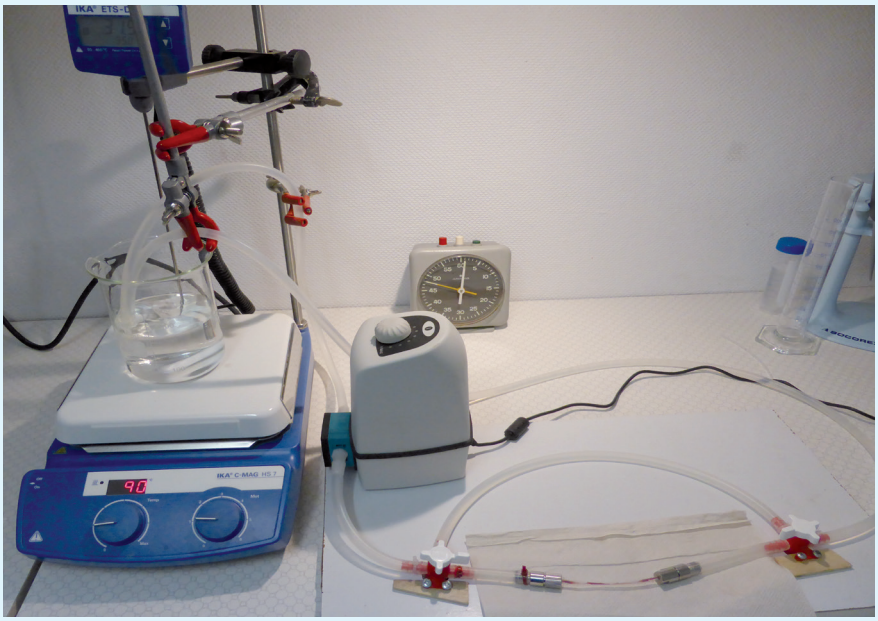

Figure 1: Test setup

* DGKH (German Society of Hospital Hygiene), DEGEA (German Society of Endoscopy Nurses and Associates), DGSV (German Society of Sterile Supply), DGVS)German Society for Digestive and Metabolic Diseases) and AKI (Working Group Instrument Preparation)

tor temperature. Following contamination the tube segments were placed on a rack and conditioned in a desiccator over saturated potassium carbonate solution at 30°C in a heating cabinet for 24 hours.

After each tube segment was placed in the circulation system under specific conditions (detergent concentration, temperature and exposure time), it was then withdrawn, rinsed with 2 ml water of the highest purity class using a syringe and then purged with air. Each tube was then cut into between 20 and 25 segments of 4-5 mm and transferred to a centrifuge test tube (Article AN76.1, Carl Roth). Following the addition of 3 ml 1% SDS solution at pH 11 each centrifuge test tube was vortexed four times for 15 seconds at 10 minute intervals after which one aliquot was tested for the presence of protein.

Since for detergents containing as an ingredient primary amines the OPA method would produce incorrect results, the modified Roti® Quant universal BCA method (Article 0120.1, Carl Roth, Karlsruhe) with photometric measurement at 503 nm was used. This was endowed with adequate sensitivity and good linearity. The limit of quantification (LOQ) was around 4.0 µg protein (BSA) for each ml 1% aliquot of SDS solution.

The following detergents were used (characterization based on information from the manufacturer):

A: Mildly alkaline detergent for EWDs composed of anionic and non-anionic surfactants, enzymes
B: Neutral detergent for a specific EWD process composed of non-anionic surfactants, enzymes
C: Mildly alkaline/enzymatic detergent for WDs and EWDs composed of non-anionic and amphoteric surfactants, enzymes
D: Detergent for WDs and EWDs composed of alkali donors, non-ionic and anionic surfactants, enzymes
E: Mildly alkaline detergent for EWDs composed of non-anionic surfactants, enzymes

Results

To determine the recovery rate the amount of protein in 20 µl blood was first measured. To that effect, 20 µl heparinised and reactivated sheep blood was transferred directly with the Hamilton syringe to 5 ml 1% SDS solution at pH 11, of which one aliquot was subjected to protein measurement. Five independent test runs yielded a mean value of 2730 µg protein with standard deviation of 78 µg. Next, using the Hamilton syringe, the tube segment was contaminated with 20 µl heparinised and reactivated sheep blood; after conditioning the tube segments were cut into small pieces, extracted and one aliquot subjected to protein measurement as described under Materials and Methods. Ten independent test runs yielded a mean value of 2725 µg protein with a standard deviation of 197 µg. That thus corresponded to a recovery rate of 99.8 %.

To ensure that for the experiments conducted with water or with detergents under different conditions the significance levels of the results would be adequately high, each test condition was investigated while using five contaminated tube segments as PCDs. That helped to balance out any irregularities during pipetting, blood distribution, etc.

First, cleaning with water alone as well as with detergents A and B at a concentration of 0.5% at 35°C and with 3-minute exposure time was tested. These are the default conditions in commercially available EWDs. Next, changes in the cleaning efficacy on increasing the temperature to 45°C with the same 3-minute exposure time were investigated. Cleaning and disinfection processes that include a thermal disinfection step operate as a rule at a temperature of around 55°C in the cleaning step. Hence, testing was also performed at that temperature to establish whether this would produce better cleaning results for the PTFE tubes. The results of these test series are summarized in Table 1.

Since these experimental series revealed that the more suitable temperature for protein removal was 45°C, detergents C, D and E were tested only at that temperature. Residual protein values below the limit of quantification of 12 µg per tube segment were obtained for all three detergents.

Since many EWDs are operated with a 5-minute exposure time in the cleaning step, all detergents were also tested at 45°C with the longer 5-minute exposure time. The results are presented in Table 2.

Discussion

Cleaning the channels of flexible endoscopes makes special demands on the process chemicals and process parameters used in EWDs. The test set-up described in this paper is eminently suitable for testing the cleaning efficacy. While fluctuations were noted with regard to the blood distribution, flow rate, temperature as well as for rinsing and sampling, which together have a cumulative effect, by using five PCDs per test condition that effect was adequately balanced out or compensated for to permit good assessment of the cleaning efficacy.

The internal surface area of the tube segments was 6.3 cm². With a guide value of 3 µg/cm² that gives an acceptance value of 18.9 µg per tube segment. That guide value is specified in the *Guideline for validation and routine monitoring of automated cleaning and thermal disinfection processes for medical devices,* compiled by the DGKH, DGSV and AKI as well as in the *Guideline for validation of manual cleaning and manual chemical disinfection of medical devices*, compiled by the societies DGKH, DGSV and AKI and is expected to also be specified in the forthcoming standard ISO EN 15883-5 n [6, 7, 8].

The standard parameters specified for the cleaning step at 35°C with 3-minute exposure time in a commercially available EWD (Table 1) are apparently not suitable for assuring effective and appropriate cleaning. While the results obtained for both detergents were markedly better than those achieved with water alone, the results achieved with detergent B, which is marked by actual EWD supplier, were above the acceptance value for four out of five PCDs. A further critical aspect here is that this supplier has, by invoking the type test, declared the cleaning step parameters as unchangeable. That casts doubt on the method used to assess the cleaning efficacy in accordance with ISO EN 15883 Part 1 and Part 4 during the type test. Even for detergent A, which assured markedly better cleaning efficacy, the residual protein quantities for some PCDs were just below the acceptance value. That must be viewed in a critical light when applied to real endoscopes with aged PTFE channels.

On increasing the test temperature to 45°C while retaining the 3-minute exposure time, the residual protein

quantities for detergents A were below the limit of quantification of around 12 µg per PCD in all cases. Likewise, the performance of detergent B was markedly better at 45°C than at 35°C, but there was greater fluctuation of the results and in one out of five PCDs the residual protein value was still above the acceptance value. A further increase in the cleaning temperature to 55°C, as customarily used in WDs with thermal disinfection processes, led to poorer results for the PTFE tube segments both on using water alone and with detergent B than those achieved at 45°C. Detergent B even appeared to reinforce protein fixation due to incipient denaturation. The optimal temperature for cleaning PTFE tubes is apparently around 45°C. That was confirmed by the results obtained with the three other detergents C, D and E at 45°C and with 3-minute exposure time, all achieving results below the limit of quantification. Prolongation of the exposure time of cleaning at 45°C to 5- minute exposure time (Table 2) did not improve the cleaning results on using water alone, however, good results were obtained for detergents, including B.

The decisive question now is whether that is also sufficient for PTFE channels of flexible endoscopes harbouring real-life soils after use and in view of the continuous creation of products that meet the requirements. That question can only be answered by means of performance qualification carried out under field conditions with real endoscopes. However, unlike performance qualification as practised for washer-disinfectors (WDs), to date this has not been done for endoscope washer-disinfectors s (EWDs). Testing with 2 m PTFE PCDs is designated in the guideline as performance qualification [3]. Based on standard EN ISO 15883-1, this is not performance qualification but is merely a test aimed at creating a link to the type test, similar to the Crile clamps used to test automated cleaning processes with thermal disinfection. According to standard EN ISO 15883-1 (6.10.3), performance qualification involves the investigation after automated cleaning of (real) instruments harbouring everyday soils. Testing with PCDs shows only with a high degree of probability that the process is able to produce products that meet the specifications but that must be verified as a factual finding during performance qualification with real instruments [9].

Table 1: µg residual protein per tube segment after cleaning at 35°C, 45°C and 55°C with 3 minute exposure time

Cleaning temperature	Detergents	PCD 1	PCD 2	PCD 3	PCD 4	PCD 5	Mean value
35 °C	**Only water**	46.0	78.5	107.4	86.6	85.4	**80.8**
	A	17.0	13.5	<LOQ	12.4	15.8	**14.1**
	B	26.3	15.8	30.9	22.8	29.8	**25.1**
45 °C	**Only water**	57.7	61.2	62.4	58.9	56.6	**58.9**
	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	B	17.0	<LOQ	24.0	18.1	<LOQ	**16.6**
55 °C	**Only water**	69.9	33.1	77.3	125.0	88.3	**78.5**
	A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	B	126.3	38.0	99.3	111.6	54.0	**85.8**

<LOQ = Below the limit of quantification (≈12 µg per tube segment); PCD = Process challenge device

Table 2: µg residual protein per tube segment after cleaning at 45°C and with 5 minute exposure time

Detergents	PCD 1	PCD 2	PCD 3	PCD 4	PCD 5	Mean value
Only water	65.8	69.3	56.4	49.4	71.6	**62.5**
A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
B	<LOQ	<LOQ	<LOQ	13,1	<LOQ	**<LOQ**
C	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
D	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
E	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**

LOQ = Limit of quantification (≈12 µg per tube segment), PCD = Process challenge device

Here a major misunderstanding comes to light with regard to the guideline for validation of EWD processes. What has been omitted in the guideline is specification of a tried and tested method for sampling and quantification of residual proteins in the channels of endoscopes harbouring real everyday soils after cleaning. There is an urgent need to work on this.

The PCDs used here were new, undamaged PTFE tube segments as also used in the published method for verification of the cleaning performance of EWDs. In reality the biopsy channels of flexible endoscopes will have been subjected to repeated passage of biopsy forceps and to cleaning with a brush causing aging of the internal channel surfaces. How, for example, minor damage to the internal surfaces impacts cleaning will now also be investigated.

References

1. DGKH, DEGEA, DGSV, DGVS, AKI: Leitlinie zur Validierung maschineller Reinigungs-Desinfektionsprozesse zur Aufbereitung thermolabiler Endoskope. Zentr Steril 2011; 19 (Suppl.3):1–72.
2. Wehrl M, Kircheis U: Method for Testing the Cleaning Efficacy of Washer-Disinfectors for Flexible Endoscopes. Zentr Steril 2011; 19 (5): 357–361.
3. Wehrl M: Control of the cleaning efficacy during performance qualification of EWD processes using test pieces according to Annex 8. Zentr Steril 2016; 24 (4): 220–225.
4. Wie Q et al.: Wechselwirkungen von Proteinen mit Polymerbeschichtungen und Biomaterialien. Angew. Chem. 2014; 126: 8138–8169.
5. Zardeneta G et al.: Protein interactions with particulate Teflon: implications fort he foreign body response. J Oral Maxillofac Surg 1996; 54 (7): 873–878.
6. Guideline compiled by DGKH, DGSV and AKI fort he validation and routine monitoring of automated cleaning and thermal disinfection processes for medical devices. Zentr Steril 2017; 25, Supplement (5th Edition)
7. Guideline for Validation of Manual Cleaning and Manual Disinfection of Medical Devices. Zentr Steril 2013; 21, Supplement
8. Martiny H: Überarbeitung der ISO TS 15883-5 – Was erwartet uns? Vortrag DGSV-Kongress 2018, Fulda
9. Kromidas S: Validierung – wozu eigentlich? Aseptica 2004; 10 (1): 3.