Original Article

Influence of the eluent on the microorganism recovery rate in endoscope test pieces

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Abstract

Microbiological evaluation of reprocessed heat-sensitive (thermolabile) flexible endoscopes after use under real, everyday conditions (real-life instruments) is carried out, e.g. in accordance with Annex 10 to the guideline compiled by the DGKH*, DEGEA, DGSV, DGVS and AKI, for performance qualification of automated reprocessing processes for heat-sensitive flexible endoscopes as well as during periodic routine checks to assure the reprocessing quality of these medical devices. Among the tasks performed for these investigations, rinse samples are taken from all endoscope channels and evaluated to determine the total number of microorganisms and the presence of any relevant potential pathogens. Elution of the endoscope channels is performed in accordance with the guideline using 0.9% sodium chloride (NaCl) solution. To investigate whether the composition of the eluent influenced the microorganism recovery rate, comparative systematic tests were conducted using the test piece/process challenge device (PCD) model described in Annex 9 to the guideline (PTFE tube, contaminated with reactivated, coagulated sheep blood and *Enterococcus faecium*). Using sodium chloride (NaCl), DNP, FHM and T+Thio solutions, the recovery rates were determined in nine participating laboratories. The results did not show any statistically significant differences between the various eluents, and the mean difference in the recovery rates did not exceed 0.51%. Based on the findings obtained with test pieces, the composition of the eluents used in this study had no influence on the microorganism recovery rate.

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

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1 Introduction

Efficacy testing of automated reprocessing processes for heat-sensitive flexible endoscopes during performance qualification of automated endoscope washer-disinfectors (EWD) includes determination of the process efficacy using test pieces as well as evaluation of reprocessed real-life instruments (flexible endoscopes harbouring soils after clinical use). In general, automated reprocessing in EWDs comprises the process steps precleaning, cleaning, intermediate rinse followed by chemothermal disinfection mainly with glutaraldehyde or peracetic acid-based disinfectants as well as final rinse steps to remove the disinfectant. Disinfection efficacy is determined by the i) contact time, ii) temperature, iii) disinfectant concentration and iv) chemical nature of the disinfectant substance provided that the disinfectant solution has unimpeded access to all surfaces and that the magnitude of the organic or inorganic soils present on the surface of the endoscopes or in the disinfectant solution is not so great as to inactivate the disinfection component (neutralization). Unlike in a thermal disinfection process where the efficacy is determined parametrically by means of the temperature and contact time (holding time), in a chemothermal disinfection process the efficacy is demonstrated using test pieces with a defined test organism count (biomonitor) used for process control purposes.

Pursuant to the German Medical Device Operator Regulation (MPBetreibV) [1], all medical device reprocessing processes must be validated. In Germany, the "Guideline for validation of auto-

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- heat-sensitive flexible endoscopes
- Annex 9 test pieces
- test organisms
- elution
- recovery rate

mated cleaning and disinfection processes for reprocessing heat-sensitive endoscopes", compiled by the DGKH, DEGEA, DGSV, DGVS and AKI [2], serves as guidance to validation of automated endoscope reprocessing processes in EWDs that comply with DIN EN ISO 15883. Microbiological sampling of reprocessed real-life instruments is set out in the KRINKO/BfArM Recommendation* [3] as well as with precise details in Annex 10 [4] to the guideline and is conducted for performance qualification (PQ) as well as for routine checks. Testing as per Annex 10 entails swab sampling of predefined critical endoscope components (distal end, if necessary the Albaran lever recess, sites that are particularly hard to access) using swabs as well as obtaining liquid/flushing samples from each channel amenable to flushing. Therefore, approx. 25 ml 0.9% sodium chloride solution (NaCl solution) is injected with a sterile disposable syringe into the respective channel and 20 ml of the eluate emerging from the distal end is collected. If the presence of disinfectant residues in the eluted channels cannot be ruled out, a suitable neutralizing agent must be added to the eluate collected. The microorganism count is determined through membrane filtration of a partial volume of the eluate, while other partial volumes of eluate are used for selective isolation of hygiene-relevant microorganisms (groups) (*Enterobacteriaceae, Pseudomonas aeruginosa, Staphylococcus* sp., streptococci, faecal streptococci, *Mycobacteria* sp., *Legionella* sp.).

Currently, the only means of microbiological testing of endoscope channels involves elution of any residual, viable microorganisms from the channels and their subsequent isolation in cultures. A high recovery rate following elution is a precondition for quantitative assessment of microorganisms. Because of the elution methodology applied in practice, the recovery rates are always less than 100% since complete recovery is not possible. However, if the recovery rates are well below 100% this leads to false-negative evaluation results and to underestimation of the actual microbial count (bioburden).

Various studies posit that elution of reprocessed real-life instruments with NaCl solution gives rise to lower recovery rates than other eluents [5–9], while other studies suggest that the composition of the eluents does not exert any influence on the recovery rate [10–12]. Because of the contradictory findings there has to date not been any consensus on whether the composition of various eluents influences the microorganism recovery rate from endoscope channels.

The *Guideline Group* responsible for formulation of the above guideline and composed of representatives of the participating societies commissioned the Methods Group 2.0, which was set up in May 2018, to systematically investigate the suitability of various eluents by conducting standardized comparative testing in nine participating laboratories.

To underpin comparative testing for systematic determination of the microorganism recovery rates from endoscope channel geometries in all participating laboratories, the established endoscope tubular test piece model used in Annex 9 to the guideline [13], based on DIN ISO/TS 15883-5:2004, Appendix I [14], was used. These test pieces consist of a 2 m long Teflon (PTFE) tube with an internal diameter of 2 mm, which serves as an accepted surrogate to reflect the geometry of endoscope channels. The test soil is composed of a defined quantity of reactivated, coagulated sheep blood for each test piece, which is mixed with a defined concentration of the test organism *Enterococcus faecium* (*E. faecium*, DSM 2146), hence, the test organism count for each test piece was known. Using these Annex 9 test pieces, the recovery rates of the test organisms they contained were determined by means of elution with the four different eluents i) NaCl solution ii) DNP solution, iii) FHM solution and iv) T+Thio solution[5–8].

The Methods Group 2.0 was coordinated by Dr. Birgit Kampf (delegated by the endoscope manufacturers' group), PD Dr. Holger Biering (delegated by the German Society of Endoscopy and Imaging Procedures [DGE-BV]) and Dr. Markus Wehrl (delegated by the German Society for Hospital Hygiene [DGKH]). The Methods Group was composed of the following participants (in alphabetical order): Chemische Fabrik Dr. Weigert GmbH & Co. KG, represented by Graduate Biologist Verona Schmidt; Hücker & Hücker GmbH, represented by Dipl.-Biol. Maciej Dabrowski; HYBETA GmbH, represented by Dirk Diedrich and Dr. Edyta Stec; HygCen Germany GmbH, represented by Dr. Oliver Riebe; Lysoform Dr. Hans Rosemann GmbH, represented by Dr. Thorsten Schwemmer-Cordes; SAL-GmbH, represented by Pia Wehnes and Dr. Kerstin Kruse; Simicon GmbH, represented by Toni Seis; SMP GmbH, represented by Klaus Roth, Dr. habil. Ludger Schnieder and Beate Dölker; DGKH, represented by Prof. Dr. Heike Martiny; Valitech GmbH & Co KG, represented by Dipl.-Ing. (FH) Daniel Geyer and M.Sc. Marc Plevschinski, Association for Applied Hygiene (VAH) and Bonn University, represented by Dr. Jürgen Gebel and Dr. Stefanie Gemein; wfk – Cleaning Technology Institute e.V., represented by Dr. Markus Wehrl.

The order in which the results of the test laboratories is presented does not correspond to the alphabetical order of the participating test laboratories.

2 Materials and Methods

2.1. Annex 9 test pieces

The materials and methods employed for production, elution and evaluation of the Annex 9 test pieces are described in Annex 9 [13] to the guideline [2].

2.2 Eluents

- NaCl solution composed of 0.90% NaCl in H_2O_{dd}, in accordance with Annex 9 [13].
- FHM solution composed of 0.10% tryptone; 0.10% Tween 80; 0.43% NaCl; 0.36% KH_2PO_4; 0.72% Na_2HPO_4 * 2 H_2O in H_2O_{dd}; pH = 7.0 ± 0.2, in accordance with [5], with reference to [15, 16].
- DNP (Diluent Neutralizing Pharmacopoeia) solution composed of 0.10% tryptone; 3.0% Tween 80; 0.30% lecithin from hen egg; 0.10% L-histidine hydrochloride; 0.43% NaCl; 0.36% KH_2PO_4 and 0.72% Na_2HPO_4 * 2 H_2O in H_2O_{dd}; pH = 7.0 ± 0.2, in accordance with [5], with reference to [16].
- T+Thio solution composed of 3.0% Tween 80; 0.30% lecithin from hen egg; 0.10% L-histidine hydrochloride and 0.50% $Na_2S_2O_3$ in H_2O_{dd}; in accordance with [5–8].

*KRINKO/BfArM Recommendation: Hygiene requirements for processing medical devices, jointly compiled by the Commission for Hospital Hygiene and Infection Prevention at the Robert Koch-Institute (KRINKO) and the Federal Institute for Drugs and Medical Devices (BfArM)

3 Results

To determine the recovery rates using the four different eluents NaCl solution, FHM solution, DNP solution and T+Thio solution, eight participating laboratories (laboratory A, B, C, D, F, G, H, I) each produced n = 12 test pieces as per Annex 9, while laboratory E used n = 42 test pieces. The concentration of test organisms contained in the test soils was between 1.55 x 10^9 and 7.35 x 10^{10} / ml. The number of test organisms used in each test piece was determined in accordance with Annex 9 for each individual test piece and was ≥ 10^9 test organisms/test piece. Only in one laboratory (laboratory C) did one single test piece contain only 8.98 x 10^8 test organisms/test piece. Table 1 presents a summary characterization of the test pieces used.

For elution with each of the four eluents n = 3 test pieces were used in each of eight laboratories (laboratory A, B, C, D, F, G, H, I). Laboratory E used n = 9 test pieces for each of eluents FHM solution, DNP solution and T+Thio solution and n = 15 test pieces for the NaCl solution. Elution was effected through injection of 50 ml of the respective eluent into a test piece as described in Annex 9. The eluates were collected and homogenized by agitation while adding 12 g steam-sterilized glass beads [13] and the number of eluted test organisms was determined by plating a decadic dilution series onto Kanamycin Aesculin Azide agar (KAA agar). Table 2 presents the recovery rates (RR_B) identified for elution with NaCl solution, which on average were 2.01 ± 2.14% (n = 39).

Figure 1 illustrates the recovery rates obtained by the laboratories using the eluents NaCl solution, FHM solution, DNP solution and T+Thio solution. The recovery rates (RR_B, see Tab. 2) identified for elution with NaCl solution were used as relative recovery rates (RRR_B) and set at 100% (baseline) to provide for systematic comparison of the elution effect of the different eluents and to compensate for any methodological differences arising in the respective laboratories. Hence, the relative recovery rates (RRR_B) presented for the eluents FHM solution, DNP solution and T+Thio solution clearly show the percentage differences in the various recovery rates compared to those obtained for elution with NaCl solution in the respective laboratory.

Compared to the reference eluent NaCl solution (baseline) with a mean relative recovery rate of RRR_B = 100% (minimum: 100%; maximum: 100%; n = 39), for elution with FHM solution mean relative recovery rates of RRR_B = 81.0% (minimum: 21.3%; maximum: 177%; n = 33) were obtained, for DNP solution mean rates of RRR_B = 76.5% (minimum: 15.8%; maximum: 181%; n = 33) and for T+Thio solution mean rates of RRR_B = 112% (minimum: 19.0%; maximum: 179%; n = 33) were identified, see Fig. 1.

Compared to the mean absolute recovery rate of RR_B = 2.01% (n = 39) observed for NaCl solution, the mean absolute recovery rate was RR_B = 1.53% for FHM solution, RR_B = 1.62% for DNP solution and RR_B = 2.04% for T+Thio solution.

For statistical evaluation the recovery rates (RR_B) identified by the various laboratories for each eluent (n = 3–15) were added together and the arithmetic mean was calculated. The mean scores obtained by the nine laboratories for each of the four eluents were summarized in each case as a data population and the four data populations for the four eluents were analysed with the Kruskal-Wallis test (IBM SPSS Statistics, Version 26). The α-error was set at α = 0.05. Statistical evaluation demonstrated that there was no significant difference between the four eluents with regard to the recovery rate ($H_{(3)}$ = 1,252, p = 0,741).

4 Discussion

Validation of reprocessing processes for heat-sensitive endoscopes in an EWD comprises, first of all, performance qualification with verification of both

Table 1: Characterization of the test pieces used with regard to the concentration of the test organisms contained in the test soil [test organisms / ml blood] and the number of test organisms injected into each test piece, shown as arithmetic mean (MV) ± standard deviation (SD) as well as minimum (MIN) and maximum (MAX). n.a.: No individual values communicated.

Laboratory	Number of test pieces [n]	Test organisms / ml blood	Test organisms / test piece		
			MV ± SD	MIN	MAX
A	12	2.95 x 10^9	1.41 ± 0.15 x 10^9	1.18 x 10^9	1.68 x 10^9
B	12	2.25 x 10^9	4.58 ± n. a. x 10^9	n. c.	n. c.
C	12	1.55 - 2.55 x 10^9	1.70 ± 0.57 x 10^9	8.98 x 10^8	2.77 x 10^9
D	12	1.80 - 3.40 x 10^9	1.85 ± 0.60 x 10^9	1.21 x 10^9	3.14 x 10^9
E	42	2.25 - 7.00 x 10^9	4.24 ± 1.62 x 10^9	1.69 x 10^9	8.00 x 10^9
F	12	7.80 - 8.90 x 10^9	5.71 ± 0.61 x 10^9	4.68 x 10^9	6.72 x 10^9
G	12	1.37 x 10^{10}	9.26 ± 1.46 x 10^9	7.52 x 10^9	1.24 x 10^{10}
H	12	7.35 x 10^{10}	4.06 ± 0.12 x 10^{10}	3.84 x 10^{10}	4.23 x 10^{10}
I	12	3.60 x 10^9	2.48 ± 0.45 x 10^9	1.98 x 10^9	3.31 x 10^9

the cleaning performance and overall process performance – as a combination of the cleaning and disinfection performance – using the process controls described in Annex 8 [17] and Annex 9 [13] of the aforementioned guideline. These process controls entail the use of a test soil of defined quality and quantity containing a defined test organism count and permitting verification of the cleaning efficacy and overall process efficacy.

The success of the reprocessing outcome must also be verified by testing instruments harbouring soils after clinical use (real-life instruments) (end product control). The quantity and composition of soils and microorganisms adhering to real-life instruments will vary greatly in accordance with the patient and the nature and intensity of instrument deployment. Hence, a negative microbiological result for reprocessed real-life instruments does not give any insight into the effectiveness of the reprocessing processes. However, detection of a single viable pathogen on or in a reprocessed endoscope is evidence that the reprocessed medical devices do not meet the requirements set out in the KRINKO/BfArM Recommendation [3].

Microbiological testing of real-life instruments

Microbiological testing of reprocessed endoscopes aimed at detection of any viable microorganisms is based on the classic microbiological culture methods using appropriate universal and selective growth media. The method described in Annex 10 [4] to the guideline entails swab sampling of critical endoscope components that are difficult to access as well as sampling of channel systems. That calls for elution with a suitable eluent of any remaining viable microorganisms from the geometrically complex channels and culture on appropriate growth media. To counter the growth-inhibiting effects of disinfectant residues, suitable neutralizing agents must be added to the eluate if the presence of such disinfectant residues in the channels at the time of elution cannot be ruled out. Hygienic evaluation of reprocessed endoscopes can be problematic following inadequate elution, i.e. if the recovery rates after elution are well below 100%, since only that subpopulation of microorganisms

Table 2: Test organism recovery rates (RR_B [%]) identified in the various laboratories and arithmetic mean calculated by all nine laboratories for test piece elution with 0.9 % NaCl solution in accordance with Annex 9 [13], illustrated as arithmetic mean (MV) ± standard deviation (SD).

Laboratory	Number of test pieces [n]	RR_B [%] for elution with NaCl solution MV ± SD
A	3	2.66 ± 0.77
B	3	5.87 ± 3.70
C	3	0.31 ± 0.15
D	3	1.79 ± 1.21
E	15	1.15 ± 0.88
F	3	1.53 ± 0.19
G	3	1.51 ± 1.69
H	3	6.15 ± 0.92
I	3	0.61 ± 0.01
MEAN	**39**	**2.01 ± 2.14**

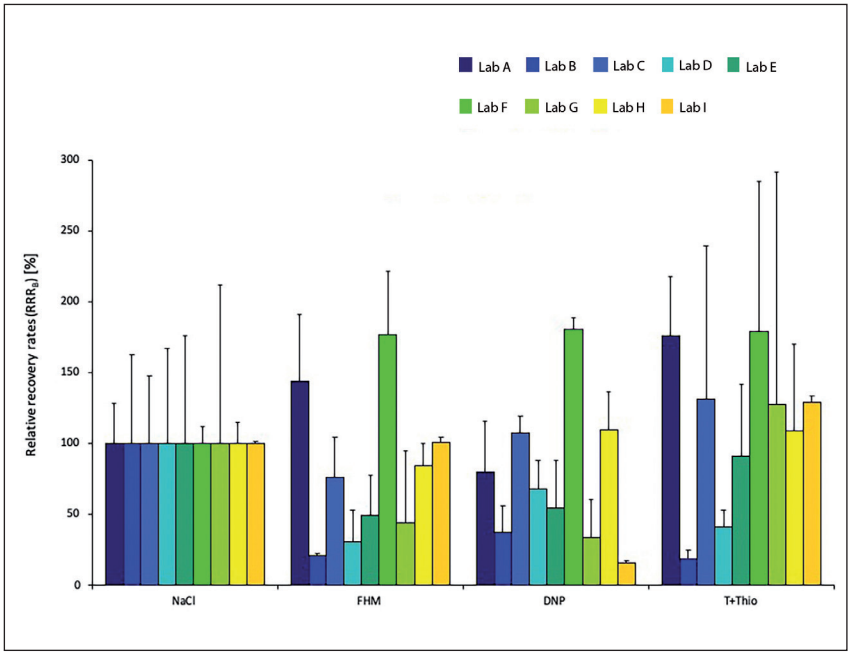

Figure 1: Relative recovery rates (RRR_B) for elution of Annex 9 test pieces in nine laboratories using the eluents NaCl solution, FHM solution, DNP solution and T+Thio solution. The recovery rates (RR_B) for elution with 0.9% NaCl solution (see Tab. 2) were used as a reference value and set at RRR_B = 100% (baseline). The recovery rates identified in each laboratory for elution with FHM, DNP and T+Thio solution were compared with that value and deviations were calculated as a relative recovery rate (RRR_B). In laboratories A, B, C, D, F, G, H, I, n = 3 test pieces were eluted with each eluent, while in laboratory E n = 15 test pieces were eluted with NaCl solution and n = 9 test pieces in each case with FHM, DNP and T+Thio solution. The lines above the respective bars denote the standard deviations (SD).

that can be flushed out can be quantitatively assessed and evaluated. Therefore, evaluation of the microbial count based on false-negative test results cannot be deemed as safe in the context of the currently recommended test method.

Recovery rates

Quantification of the microorganism recovery rate from the endoscope channels of real-life instruments is a topic of current debate and investigation. Corroborated and reproducible results for the microorganism recovery rate from channel geometries are only available for well-characterized endoscope test pieces using a defined test soil and defined test organism count in accordance with Annex 9 [13]. Elution based on injection of 50 ml NaCl solution yields recovery rates of 0.1–2%, for these test pieces, as demonstrated by numerous studies and by comparative testing previously carried out by the Methods Group [11,12,18–20]. Compliance with this recovery rate is a binding criterion for specification and quality assurance of test pieces produced in accordance with Annex 9 [13] since it demonstrates that these test pieces make defined and stringent demands on a reprocessing process.

Using *P. aeruginosa* biofilm artificially cultivated in PTFE tubings, Aumeran *et al.* demonstrated that higher recovery rates were obtained with Letheen Broth, of complex composition, compared to NaCl solution or water. Likewise, sampling of reprocessed real-life instruments after elution with Letheen Broth yielded a higher microorganism recovery rate [9]. Richard *et al.* carried out tests on reprocessed real-life instruments. Using repeated (duplicate) sampling in accordance with ISO 11737-1 [21] followed by calculations, the recovery rate of microorganisms eluted from the channels was estimated. The eluents 0.9% NaCl solution, DNP solution, FHM solution and T+Thio solution were used for elution of the real-life instrument channels. Two consecutive channel elution steps were performed, yielding recovery rates of approx. 92% for T+Thio solution, approx. 87% for DNP solution, approx. 77% for FHM solution and approx. 2% for NaCl solution [5–8]. Because it yielded the highest recovery rate, Pineau *et al.* used T+Thio solution as the standard for subsequent tests. Cattoir *et al.* used for one study PTFE tubings harbouring either *Klebsiella pneumoniae* or a biofilm with *P. aeruginosa, K. pneumoniae* and *Staphylococcus epidermidis*. The eluents used were Neutralizing Pharmacopoeia Diluent (NPD) or physiologic saline (NaCl solution) alone or in combination with disposable endoscopic brushes or the Pull Thru® System (manufactured by F.R. GALANTAI MANUFACTURING LTD., New Zealand). No significant differences were identified between the recovery rates obtained with any of the four elution methods investigated [10].

The Methods Group 2.0 results presented here for nine laboratories that used the four different eluents NaCl solution, DNP solution, FHM solution and T+Thio with test pieces as described in Annex 9 do not show any significant differences in the recovery rates. The results obtained by the Methods Group 2.0 for test pieces differ from those identified by Richard *et al.* [5–8] for reprocessed real-life instruments. That can be explained by the fact that the Methods Group 2.0 tests with test pieces containing a defined test organism count and a defined test soil were performed for test pieces that had not been reprocessed. By contrast, Richard *et al.* tested the most diverse reprocessed real-life instruments which are thought to have harboured a broad range of soils and different microorganism counts. Since the microorganism count (bioburden) actually present after reprocessing was not known, the recovery rate had to be determined through repeated, duplicate, elution and then calculated. Furthermore, any disinfectant residues in the reprocessed endoscopes can lead to different results compared to those obtained for the test pieces. The eluents FHM solution, DNP solution and T+Thio solution contain tryptone, Tween 80, lecithin, histidine and histidine hydrochloride or sodium thiosulphate which, as neutralizing agents, are commonly used for neutralization of disinfectants. Accordingly, it can be assumed that any disinfectant residues present will have been effectively neutralized when using these three eluents for reprocessed real-life instruments. Conversely, since NaCl solution has no neutralizing properties, in accordance with Annex 10 [4] to the guideline neutralizing agents must be added to the eluate collected.

It does not appear plausible that organic substances such as tryptone, Tween 80, lecithin, histidine or sodium thiosulphate will exert a positive influence on the suspendability, i.e. detachment and elution of microorganisms from the geometrically complex endoscope channel lumens because it is assumed that microorganisms are found embedded in residual soils on the channel surface. Since other than Tween 80 none of the well-known neutralizing agents is endowed with surface activity, and even the surface activity of Tween 80 does not exceed the activity of surfactants in instrument cleaners, it is not plausible to assume they will be better at detaching residual soils and microorganisms.

The mean recovery rates (RR_B) for all four eluents investigated on using test pieces contaminated with coagulated sheep blood and *Enterococcus faecium* as per Annex 9 differed by 0.51% (maximum: T+Thio solution with RR_B = 2.04 ± 2.29% (n=33) and minimum FHM solution with RR_B = 1.53 ± 1.68% (n=33)). Based on the results obtained, it can be assumed that the composition of the eluents used for elution of Annex 9 test pieces that had not been reprocessed had no influence on the microorganism recovery rate from channel geometries.

Outlook

Current and future studies by the Methods Group 2.0 aim to increase the recovery rate for microorganism elution from channel geometries using alternative flushing techniques and greater mechanical action [22, 23] based on a flush-brush-flush method.

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