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Elution of working channels with the flush-brush-flush-method for microbiological testing of reprocessed endoscopes

Part 1: Description of the method and microbiology results of the field study

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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:
Wehrl M, Barone P, Biering H, Brill F H H, Dabrowski M, Diedrich D, Gebel J, Gemein S, Geyer D, Halvarsson A, Hücker B, Kampe A, Kampf B, Kruse K, Lenz J, Martiny H, Orschel U, Plevschinski M, Riebe O, Roth K, Schilberg V, Schmidt V, Schnieder L, Schwemmer-Cordes T, Seis T, Stec E, Uhlig C, Wehnes P. Elution of working channels with the flush-brush-flush-method for microbiological testing of reprocessed endoscopes – Part 1: Description of the method and microbiology results of the field study. Zentr Steril 2022; 30 (5): 272–277.

Manuscript data:
Submitted:
29 August 2022
Accepted:
29 September 2022

1 Introduction

In accordance with the hitherto valid Annex 10 [1] of the first edition of the *Guideline for Validation of Automated Cleaning and Disinfection Processes for Reprocessing Heat-Sensitive Endoscopes*, compiled by the DGKH, DEGEA, DGSV, DGVS and AKI* [2], microbiological testing of reprocessed endoscopes (product control) is carried out by swab sampling of critical external areas and flush sampling of all endoscope channels. The basis for this description is Annex 8 of the KRINKO/BfArM** Recommendation *Hygiene Requirements for Reprocessing Medical Devices* [3]. For flush sampling 25 ml of sterile 0.9 % sodium chloride solution (NaCl solution) was injected into each of the channels present and 20 ml was separately collected from the distal tip. The swab and flush samples were evaluated using microbiology culture methods to obtain the number of eluted microorganisms (quantitative testing) and by using selective nutrient media for detection of hygienically relevant indicator organisms (qualitative testing). Elution of residual viable microorganisms from the channels through flush sampling followed by detection in cultures is currently the only established method in Germany for microbiological testing of endoscope channels that are not directly accessible.

When testing the microbiological condition of endoscope channels the recovery rate of the detected microorganisms is of elementary importance. The recovery rate is the quantitative measure of the proportion of detected microorganisms in relation to the number of microorganisms actually present in the test object, i.e. the respective endoscope channel. Low recovery rates result in underestimation of the actual bioburden and may lead to an unrecognised risk from undetected pathogens.

Keywords
- heat-sensitive flexible endoscopes
- working channel
- channel system
- elution
- flush-brush-flush
- overall process performance
- product control
- performance qualification, performance requalification

Scientific and technical background to the need for new elution techniques

The recovery rate of microorganism from the endoscope channels of reprocessed real-life instruments is a topic of current discussion. As regards the microbiological testing of heat-sensitive endoscopes, there are different national methods for both sampling and culturing. Whereas in some countries, including Germany, only flush sampling of all channels is used, in other countries even in the past mechanically assisted elution through the use of brushes was included in routine sampling of the working channel, for example in France where the working and suction channels were sampled using a flush-brush-flush (FBF) method for all different kinds of endoscope models and only the non-brushable channels were sampled by flushing [4]. The FBF method was also already used in the past in studies for determination of the bioburden on clinically used endoscopes [5, 6]. Likewise, methods underpinned by mechanical components were used for investigation in outbreak situations [7]: Because of the increasing reports since 2013 of transmission of multidrug-resistant pathogens via duodenoscopes [8, 9], the Food and Drug Administration (FDA) recommended in 2015, among other things, the introduction of routine microbiological testing of working channels and of the Albaran lever recess of duodenoscopes. A protocol that included the FBF method has been in place since 2018; this was drawn up by the FDA, the Centers for Disease Control (CDC) and the American Society for Microbiology (ASM) in collaboration with the duodenoscope manufacturers and other experts [10]. The test series conducted by CDC on reprocessed duodenoscopes revealed that instrument sampling involving only flush elution methods did not find any critical (facultative pathogenic) indicator microorganisms on the instruments. However, on using the FBF method it emerged that two-thirds of the tested duodenoscopes harboured critical indicator microorganisms [7], hence the FBF method recovery rate must have been higher. The FBF method was also introduced in Version 4.1 of the Dutch manual *Professional Standard Handbook Cleaning and Disinfection Flexible Endoscopes* (2017) of SFERD (Steering group for flexible endoscope cleaning and disinfection) [11]. On this basis, it was further developed by Rauwers et al. and applied in the Netherlands in a large study to investigate the reprocessing quality of duodenoscopes [12].

Already back in 2001, Dietze et al. demonstrated that in the case of brushable air/water channels contaminated with blood and *Enterococcus faecium (E. faecium)* it was possible to increase the recovery rates from 3 % to 97 % [13]. Other methodological approaches for increasing the recovery rate focused on the use of specific eluents in combination with flush methods. A sharp increase was observed in the recovery rate from the channels of reprocessed endoscopes after patient use (real-life instruments) when using eluents containing substances that have a neutralising effect on the disinfectants used compared to physiological NaCl solution [14–17].

To investigate whether the eluent composition also influenced the microorganism recovery rate when using test pieces (process challenge devices [PCD]) as per Annex 9 of the guideline [18] (PTFE tubing contaminated with reactivated, coagulated sheep blood and *E. faecium* as test organism), the Methods Group 2.0 conducted systematic tests. Within the framework of standardised comparative studies, it was possible to demonstrate that the eluent composition did not have a statistically significant influence on the microorganism recovery rate in test pieces using the above test soil [19]. From extensive systematic tests under comparative conditions within Methods Group 2.0 as well as from numerous other systematic tests, it is known that elution of very well standardised Annex 9 test pieces with flush methods yield recovery rates of between 0.1 and 2 %.

Because of these lower recovery rates yielded by flush methods, the group responsible for compilation of the *Guideline for Validation of Manual Endoscope Procedures* composed of representatives of the participating societies (*Guideline Group*) commissioned the Methods Group 2.0 set up in May 2018 to develop optimised elution methods for microbiological testing of reprocessed endoscopes after patient use, which yield higher microorganism recovery rates from the channel system.

Work carried out for method development

The work aimed at method development was carried out in stages:

- First, systematic testing of the recovery rates yielded by different elution methods for Annex 9 test pieces with a defined number of test organisms was carried out. Overall, the 12 participating laboratories of Methods Group 2.0 conducted six comparative studies for the development and characterisation of the new FBF elution method.
- In the second step, investigation and characterisation of the FBF elution method was done within the framework of a pilot study using six different endoscope working channel types with unknown microbiological status, which had been removed from real-life instruments returned for repair and made available by five endoscope manufacturers to Methods Group 2.0 for testing.
- In a final field study the FBF method was applied for sampling the working channels of n=101 endoscopes after patient use and subsequent reprocessing.

Publication of the findings

This paper now describes the flush-brush-flush (FBF) method and the microbiology results of a field study. Further results of this field study, pilot study and selected results of comparative studies previously conducted by Methods Group 2.0 will be reported in future publications.

Composition of Methods Group 2.0

Methods Group 2.0 was coordinated by Assistant Professor, Dr Holger Biering

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* 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
** 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)

(delegated on behalf of the German Society of Endoscopy and Imaging Processes [DGE-BV]), Dr Birgit Kampf (delegated on behalf of the endoscope manufacturers' group) and Dr Markus Wehrl (delegated on behalf of the German Society of Hospital Hygiene [DGKH]). The Methods Group 2.0 is composed of the authors of this publication and includes, among others, 12 laboratories offering commercial microbiology test services as well as non-commercial research laboratories, which performed the experimental tasks in the field study, pilot study and comparative studies.

2 Materials and Methods

For the practical conduct of the field study the participating laboratories of Methods Group 2.0 were instructed to use the following materials and methods:

2.1 Materials

- Consumable materials
  - Hand disinfectants
  - Sterile syringes, 50 ml, with Luer lock
  - Sterile collecting vessels, volume > 50 ml
  - Sterile scissors or side cutters for separating the brush heads
  - Individually packed new (single-use) endoscope cleaning brushes with diameter tailored to the working channel of the test endoscopes
  - Sterile glass beads with 3 mm diameter
  - Horizontal agitator with 300 rpm setting option
  - Sheep blood agar plates
  - Membrane filtration apparatus
  - Sterile membrane filter, 47 mm diameter, pore size 0.2 μm
- Eluent containing NaCl-TG (Sodium Chloride/Tween/Glycerine) Eluent for endoscopes disinfected with glutaraldehyde-based processes:
  - NaCl 8.5 g
  - Tween 80 10 g
  - Glycerine 20 g
  - Distilled water ad 1.0 l

  Using pH indicator strips and, if necessary, adding 1 M HCl or 1 M NaOH solution, the eluent was set to pH = 7.0 ± 0.2 and then autoclaved in a steam sterilization process (121 °C, 20 min).
- Eluent containing NaCl-TLH-Thio (Sodium Chloride/Tween/Lecithin/Histidine/Sodium Thiosulphate) Eluent for endoscopes disinfected with peracetic acid-based processes:
  - NaCl 8,5 g
  - Tween 80 30 g
  - Lecithin 3.0 g
  - Histidine 1.0 g
  - Sodium thiosulphate 5.0 g
  - Distilled water ad 1.0 l

  Using pH indicator strips and, if necessary, adding 1 M HCl or 1 M NaOH solution, the eluent was set to pH = 7.0 ± 0.2 and then autoclaved in a steam sterilization process (121 °C, 20 min).

2.2 Methods

2.2.1 Sampling preparation

Before sampling, the endoscope type and serial number, type of preceding reprocessing process (automated, manual, possibly with semi-automated support) and type of detergents and disinfectants used as well as the time between reprocessing and sampling were recorded. Likewise, the type of endoscope cleaning brushes used for sampling (design, diameter) was documented. The eluent was chosen in accordance with the disinfectant substance used in the preceding reprocessing process in the various institutions. Sampling had to be performed under aseptic conditions. Contamination of the test endoscope, recovered sample, endoscope cleaning brush used as well as the sample collecting vessels, e.g. through touching, had to be prevented. It was recommended that sampling be carried out on an endoscope by two people. Sampling was performed by personnel who had in advance been familiarised with the new sampling method and were routinely entrusted with hygiene checks in the medical institutions (e.g. endoscopy personnel, the designated infection control nurse) together with the Methods Group 2.0 members.

Depending on the model and length of the test endoscope, the device was freely suspended for sampling or placed on a sterile disposable underlay, so as to permit non-touch collection of the eluent from instrument parts of critical relevance for testing and without touching the underlay.

2.2.2 FBF elution of working channels

Using a sterile syringe containing 50 ml of the chosen eluent, 25 ml of the eluent was injected into the working channel port (1st flush). The syringe was connected preferably liquid-tight with the Luer lock, if present on the endoscope. If there was no Luer lock on the endoscope, the eluent was carefully injected, without splashing, with the syringe opening into the channel port. The eluent was recovered at the distal tip in a sterile collecting vessel. To avoid contamination, the wall of the sterile collecting vessel was not allowed to come into contact with the distal tip of the endoscope. Eluent residues were blown out of the channel by injecting 2 x 50 ml air. If no Luer lock was fitted, air injection had to be omitted. The eluent volume recovered in the 1st flush was documented.

Next, the brush head of an endoscope cleaning brush tailored to the working channel diameter was moistened with the chosen eluent, inserted into the working channel port and advanced as far as the distal tip (brush). To assure contamination-free introduction of the endoscope cleaning brush into the working channel, it was possible to aseptically open, for example, the packaging at one corner and insert the brush head into the channel by advancing the brush shaft while still in the bag. The brush head emerging from the distal end of the endoscope was cut off together with approx. 1 cm of brush shaft using sterile scissors or sterile side cutters and collected in a second new, sterile collecting vessel. The brush shaft remaining in the channel was carefully pulled backwards and discarded. In the case of endoscope cleaning brushes that had a second brush head at the end of the shaft, this second brush head was not pulled through the channel and not included in the evaluation, but discarded together with the retracted shaft. The detached and recovered brush head was visually inspected in the collecting vessel for possible residual soils/residues and, if possible, photographed.

This was followed by the second flush (2nd flush) while injecting the remaining 25 ml eluent from the syringe. The same procedure was used as for the first flush (1st flush). The eluate was recovered at the distal end in a second collecting vessel already containing the detached brush head. Eluent residues were blown out of the channel by injecting 2 x 50 ml air. If no Luer lock was fitted, air injection had

to be omitted. The eluent volume recovered from the brush and in the 2nd flush was documented.

2.2.3 Sample transportation

After sampling, the samples were transported as quickly as possible to the test laboratory to assure further processing within 24 h of sampling. Refrigerated transportation was used if the transportation time until laboratory processing was more than 4 h (< 6 °C).

2.2.4 Sample processing and evaluation

10 g sterile glass beads were added to each collecting vessel (3 mm diameter). The eluate was homogenized for 30 min on a horizontal agitator at 300 rpm (at recorded room temperature). The total volume of homogenized eluate was passed through a membrane filter (membrane filter diameter: 47 mm, membrane pore size 0.2 μm). The filter membrane was aseptically transferred to a sheep blood agar plate and incubated under aerobic conditions at 36 ± 1 °C. The first reading and documentation of the total number of colony-forming units (total colony count) were done after 24 ± 2 h, and the second reading after 48 ± 4 h.

3 Findings

To characterise the method, the FBF technique was applied in the field study by members of Methods Group 2.0 in 34 medical endoscopy units (hospitals, medical care centres and office-based medical practices) working in collaboration with the personnel of the respective institution. The participating medical institutions were chosen by the German Society of Endoscopy Nurses and Associates (DEGEA) and the Association of Resident Gastroenterologists (BNG). In each participating medical institution n = 3 (in one institution only n = 2) endoscopes (real-life instruments), which had been reprocessed after patient use and chosen by the institution itself, were investigated. In total, n = 101 endoscopes were inspected. The endoscope types represented were colonoscopes, gastroscopes, duodenoscopes and bronchoscopes, each from different manufacturers. Depending on the specific institution, the endoscopes were reprocessed using an automated or a manual reprocessing process and glutaraldehyde or peracetic acid. The results were made available by the members of Methods Group 2.0, and were collated and evaluated anonymously by Dr Markus Wehrl (wfk – Cleaning Technology Institute e.V.).

For the n = 101 endoscopes inspected, the following total number of colony-forming units (CFU) were counted in the eluate of the 1st flush: n = 60 endoscopes (59 % of n = 101 endoscopes) did not have any detectable microorganisms, n = 35 endoscopes (35 % of n = 101 endoscopes) had microbial counts of 1 – 20 CFU and n = 6 endoscopes (6 % of n = 101 endoscopes) had microbial counts of > 20 CFU, see Fig. 1.

For the n = 101 endoscopes inspected, the following total number of colony-forming units (CFU) were determined in the eluted brush heads (brush) in addition to the eluates from the 2nd flush: n = 36 endoscopes (36 % of n = 101 endoscopes) had no detectable microorganisms, n = 49 endoscopes (48 % of n = 101 endoscopes) had microbial counts of 1 - 20 CFU and n = 16 endoscopes (16 % of n = 101 endoscopes) had microbial counts of > 20 CFU, see Fig. 2.

By adding the total colony counts yielded by the 1st flush eluate, the eluted brush heads and the 2nd flush eluate (these were added separately for each endoscope) – which would correspond to the collection of both flush samples and the brush heads in one collecting vessel – the following results were obtained: n = 26 endoscopes (26 % of n = 101 endoscopes) had no detectable microorganisms, n = 53 endoscopes (52 % of n = 101 endoscopes) had microbial counts of 1 - 20 CFU and n = 22 endoscopes (22 % of n = 101 endoscopes) had microbial counts of > 20 CFU, see Fig. 3.

4 Discussion

This field study confirmed that on using an endoscope cleaning brush and a second flush step (brush, 2nd flush), compared with flush sampling alone (1st flush), more microorganisms were detected in a higher percent proportion of the instruments inspected. In the 1st flush sampling, no microorganisms (0 CFU) were detected on 59 % of endoscopes.

By contrast, in the subsequent sampling based on brushing and 2nd flush of the same instruments already flushed once before, the proportion of endoscopes without detectable microorganisms was only 36 %. The addition of the microbial counts of both subsequent sampling steps clearly highlights that with the combination of the 1st flush – brush – 2nd flush only 26 % of instruments had no detectable microorganisms.

For the category > 20 CFU the percent proportion of endoscopes rose from 6 % for the 1st flush to 16 % for

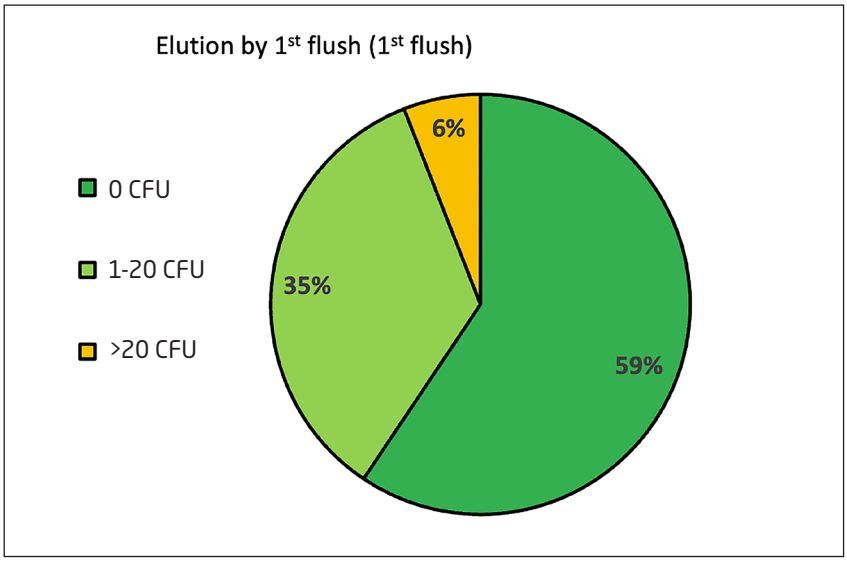

Fig. 1: Percentage distribution of the n = 101 results obtained for the total colony count from the 1st flush of the working channel, divided into the categories 0 CFU, 1 - 20 CFU and > 20 CFU per working channel.

the brush and 2nd flush and to 22 % of instruments for the addition of 1st flush, brush and 2nd flush.

The results obtained demonstrate that the microorganism recovery rate can be sharply increased by using an endoscope cleaning brush, followed by a 2nd flush. The results obtained are in concordance with the CDC investigation results [7]. The higher recovery rate reduces the risk of false-negative results because there is a greater probability of identifying inadequately reprocessed endoscopes, thus enhancing the safety of patients, users and third parties. Based on the methodological superiority of the flush-brush-flush (FBF) method demonstrated in the field study, it makes sense to replace with the new FBF method the simple (single) sampling method used hitherto to sample the channels of reprocessed endoscopes.

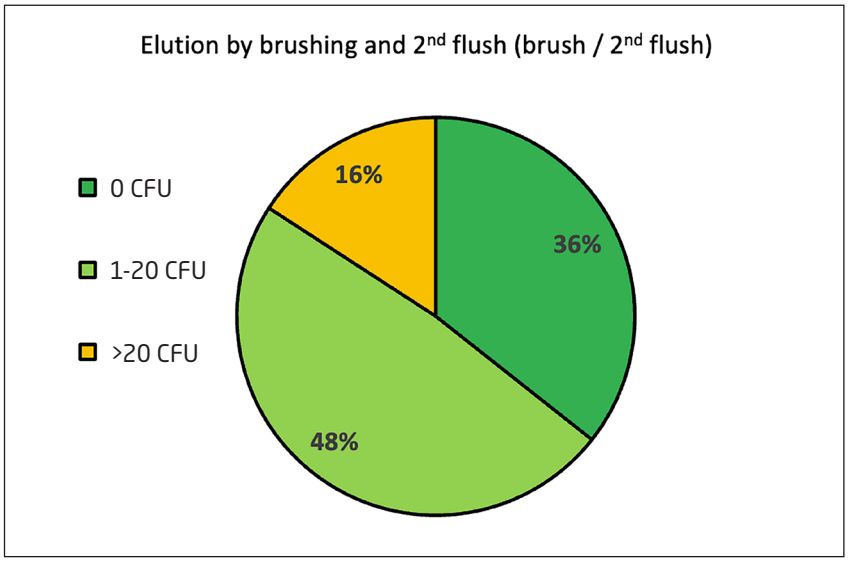

Fig. 2: Percentage distribution of the n = 101 results obtained for the total colony count from elution of the brush heads (brush) and the 2nd flush of the working channel, divided into the categories 0 CFU, 1 – 20 CFU and > 20 CFU per working channel.

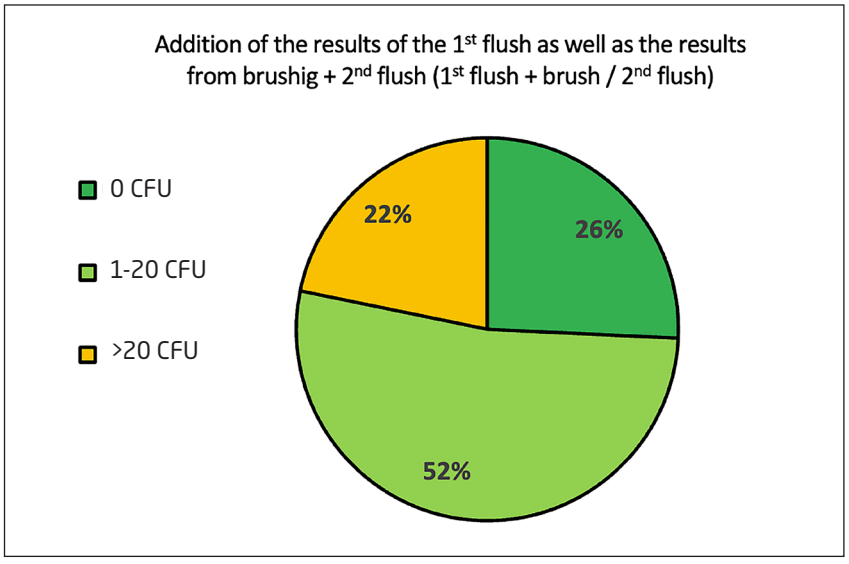

Fig. 3: Percentage distribution of the n = 101 results obtained on adding the total colony counts from the 1st flush as well as from elution of the brush heads (brush) and from the 2nd flush of the working channels, divided into the categories 0 CFU, 1 – 20 CFU and > 20 CFU per working channel.

Acknowledgements

We thank the medical institutions which made the field study possible by sampling their endoscopes. We thank Ulrike Beilenhoff (DEGEA) and Dr Markus Dreck (BNG) for appealing to and selecting the medical institutions that participated in the field study. For technical support and conduct of experimental tasks, we thank Christine Lerch and Verena Krapp (biocheck Hygienetechnisches Labor GmbH), Elena Imenova and Dörte Bünting (HygCen Germany GmbH), Per Stalinsky (Department of Hygiene and Public Health of University Hospital Bonn) and Malte Treder (SAL-GmbH). Special thanks to Eugenia Friesen, Eugenie Chartschenko and Alexander Hammermeister (wfk – Cleaning Technology Institute e.V.) for conduct of the initial orientational series of tests.

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