

ORIGINAL ARTICLE

Manual Methods Are Suboptimal Compared With Automated Methods for Cleaning of Single-Use Biopsy Forceps

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OBJECTIVE. Most reusable biopsy forceps and all of the currently available single-use biopsy forceps do not have a port that allows fluid flow down the inner tubular shaft of the device. Reusable biopsy forceps are widely used and reprocessed in healthcare facilities, and single-use biopsy forceps are reprocessed either in-house (eg, in Canada and Japan) or by third-party reprocessors (eg, in the United States). The objective of this study was to determine the cleaning efficacy of automated narrow-lumen sonic irrigation cleaning, sonication-only cleaning, and manual cleaning for biopsy forceps.

DESIGN. A simulated-use study was performed by inoculating the inner channel of single-use biopsy forceps with artificial test soil containing both *Enterococcus faecalis* and *Geobacillus stearothermophilus* at concentrations of 10^6 colony-forming units per milliliter. The cleaning methods evaluated were manual cleaning, sonication-only cleaning, and "retroflush" cleaning by an automated narrow-lumen irrigator. Bioburden and organic soil reduction after washing was evaluated. Forceps used in biopsies of patients were also tested to determine the worst-case soiling levels.

RESULTS. Only retroflush irrigation cleaning could effectively remove material from within the shaft portion of the biopsy forceps: it achieved an average reduction of more than 95% in levels of protein, hemoglobin, carbohydrate, and endotoxin. However, even this method of cleaning was not totally effective, as only a 2 \log_{10} reduction in bioburden could be achieved, and there were low residual levels of hemoglobin and carbohydrate.

CONCLUSION. The data from this evaluation indicate that manual and sonication-only cleaning methods for biopsy forceps were totally ineffective in removing material from within the biopsy forceps. Even the use of retroflush cleaning was not totally effective. These findings suggest that in-hospital reprocessing of biopsy forceps with currently available equipment and cleaning methods is suboptimal.

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Biopsy forceps used during endoscopic procedures that involve the gastrointestinal tract are currently available as reusable biopsy forceps or single-use biopsy forceps (SBFs). Both reusable forceps and SBFs are considered to be "critical" devices, according to the Spaulding classification, because they transgress the mucosal tissue and enter sterile tissue when used to perform biopsies.^{1,2,3,4} These critical devices must be sterile when used for patient procedures. Unlike other surgical medical devices used in invasive surgery that is classified as "clean," these biopsy forceps and SBFs are exposed to a high bioburden level, in addition to organic material, as a result of contact with the mucosal surface of the bowel. Although SBFs are produced and sold as single-use devices, there are healthcare centers (eg, in Canada and Japan) and third-party commercial companies (eg, in the United States) that reprocess these devices.^{5,6,7} Although the US Food and Drug Administration currently regulates the reprocessing of single-use devices, they have approved some SBFs for reprocessing by third-party reprocessors on the basis

of validated protocols.⁸ Indeed, SBFs are one of the most commonly reprocessed single-use devices in the United States, as well as in other countries.

A critical stage in the reprocessing of any medical device that has been used in a patient procedure is ensuring that it has been adequately cleaned prior to sterilization. Cleaning involves the removal of both patient-derived organic "soil" and microorganisms (ie, reduction of the bioburden). Routine cleaning is expected to effect a 3-4 \log_{10} reduction in the bioburden.^{9,10} Few published data are available regarding the expected extent of original soil removal, other than guidelines that state that the device should be visibly clean.^{1,2,9} There have been studies evaluating the ability to reduce the bioburden on SBFs;¹¹ however, there are no published studies that have used either in situ or destructive quantitative tests to evaluate the efficacy of cleaning (ie, both bioburden reduction and organic soil removal).

The objective of this evaluation was to use simulated-use testing to determine how well single-use biopsy forceps can

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be cleaned by automated sonic irrigation cleaning, sonication-only cleaning, and manual cleaning.

METHODS

Handling of the biopsy forceps for testing after cleaning and/or after sterilization was performed in a class IIB biosafety cabinet and appropriate personal protective equipment was used.

Test Soil

The test soil used was Artificial Test Soil (ATS; US patent #6,447,990, to M.J.A.), which is formulated to mimic worst-case soiling expected in gastrointestinal endoscopy procedures and allows quantitative assessment of cleaning efficacy for protein, carbohydrate, hemoglobin, and endotoxin.¹²⁻¹⁴ The ATS was stored at 4°C and was used within 1 month after its preparation.

Bioburden

The ATS was supplemented to contain approximately 10⁶ colony-forming units (cfu)/mL of *Enterococcus faecalis* (ATCC 29212) as well as approximately 10⁶ cfu/mL of *Geobacillus stearothermophilus* (ATCC 12980) spores (Presque Isle Cultures). Inoculum counts were performed to confirm the concentration of both organisms for all experiments.

Test Devices and Inoculation Procedure

The test devices were new biopsy forceps (Radial Jaw 3; Microvasive; Boston Scientific). This type of biopsy forceps has an friction-reducing sheath (Endoglide), a working length of 240 cm, an outside jaw diameter of 3.3 mm, and is for use with a biopsy channel that has a minimum internal diameter of 3.8 mm. All biopsy forceps were new and unused and were provided by Boston Scientific. The retroflush lumen adaptor from Medisafe was used for inoculation of ATS (Figure 1A), which was forced upwards through the retroflush lumen adaptor into the SBF until excess soil was noted exiting at the handle. This procedure ensured that the entire inner channel was inoculated. The inoculated SBFs were stored at room temperature for 2 hours, then cleaned using the 3 test methods to be evaluated.

Cleaning Methods Evaluated

Automated retroflush irrigation. A sonic irrigator (SI Auto; Medisafe UK, who provided the unit for this study) was used as the method of providing retroflush cleaning. The retroflush adaptor (Figure 1B) was used to connect the SBF to the port of the irrigator. This allowed enzymatic detergent as well as rinse water to be flushed up the inner tubular channel through the distal end of the SBF. Either the 5-minute or 15-minute cleaning cycle at approximately 43°C was used for this evaluation. The enzymatic detergent 3E-Zyme (Medisafe UK) was used at a dilution of 7 mL in 1 L of tap water.

Sonication and external washing only. To simulate cleaning

by sonication combined with external washing (a protocol similar to one that might be used in hospitals), the inoculated SBF was placed in the sonic irrigator but was not connected to the lumen irrigator by the retroflush adaptor. Therefore, the SBF was exposed to enzymatic detergent, sonication, and external rinsing for the same length of time as in the short (5-minute) automatic cleaning cycle.

Manual cleaning. The inoculated SBF was immersed in enzymatic detergent at the dilution recommended by the manufacturer (the same as that used in the sonic irrigator) for 5 minutes. The detergent dilution and exposure time used for the manual method were the same as that used for the sonication only and the sonic-irrigation cleaning methods. A 10-mL sterile syringe was used to flush enzyme upwards at the distal end of the SBF (20 mL total). The external surfaces of the SBF were cleaned with gauze while immersed in the enzymatic detergent. The SBF was then transferred to a basin and rinsed with tap water (repeated to provide 2 separate water rinses). This provides a simulated-use test similar to a manual cleaning process that might be used in hospitals that reprocess SBFs.

Test methods

Quantitative indirect evaluation of soil parameters and count of viable organisms. After the test SBFs were cleaned, each one was aseptically cut up into approximately 4.5-cm lengths, and the segments from each separate SBF were pooled in a 50-mL sterile test tube (Corning). Each segment stood vertically within the test tube. Once the entire length of the SBF (excluding the handle) was cut up, 25 mL of sterile, reverse-osmosis-purified water was added to the test tube. The SBF segments were completely immersed in the water. The tube containing the SBF segments was mixed by a vortex mixer for 1 minute, sonicated for 4 pulses of 5 seconds each, centrifuged at 3,500 rpm for 10 minutes at 4°C (to ensure that all lumens were perfused with liquid), and mixed by a vortex mixer for an additional 1 minute. The eluted sample was then used to determine the amount of protein (with Bradford's protein assay; Sigma Chemical), carbohydrate,¹⁵ hemoglobin (with TMB-One Blue; Biotecx Laboratories), and endotoxin (with the LAL assay; Associates of Cape Cod). The limits of detection for these assays are as follows: for carbohydrate, 5 µg/mL (125 µg per device); for hemoglobin, 5 µg/mL (125 µg per device); for protein, 0.5 µg/mL (12.5 µg per device), and for endotoxin, 0.005 endotoxin units per mL (0.125 endotoxin units per device).

Counts of viable organisms counts were performed using serial 1 : 10 dilutions and the spread plate technique. Briefly, 0.1 mL of each dilution of the sample was spread over the surface of 2 tryptic soy agar plates; one set of inoculated plates was incubated at 55°C (to detect *G. stearothermophilus*), and the other set was incubated at 35°C (to detect *E. faecalis*). All tests were performed in triplicate. The limit of detection for viable-organism counts was 10 cfu/mL (250 cfu per device).

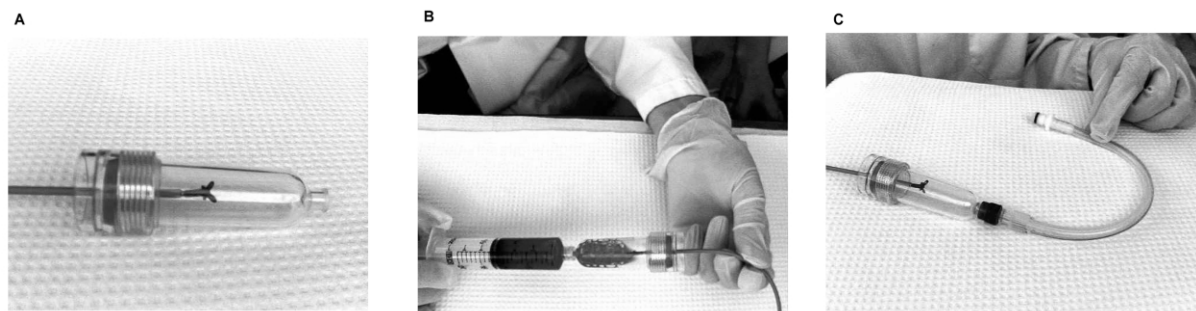


FIGURE 1. Retroflush adaptor used for single-use biopsy forceps (SBF) inoculation and for connection to the SI Auto narrow lumen cleaner. A, The neoprene gasket has a hole in the center that allows the SBF to be inserted into the chamber of the adaptor. The gasket provides a tight fit around the shaft of the SBF once it has been inserted. B, The Artificial Test Soil is injected into the retroflush adaptor (as described in Methods) with a syringe that attaches to the luer port of the retroflush adaptor. C, Retroflush adaptor used to connect SBF to the SI Auto device. The portion of the tubing adaptor that is being held in the figure is used to connect the retroflush adaptor to a fluid port in the SI Auto cleaner. The connections are tight enough that fluid is forced into the adaptor chamber and then up through the distal end of the SBF.

Bradford's *in situ* test method. Bradford's reagent (Sigma Chemical) can be perfused into the inner lumen as a direct test to determine how efficiently the protein has been removed (the reagent turns blue in the presence of protein).¹⁶ The Bradford reagent was instilled into the SBF using the retroflush lumen adaptor (Figure 1), and the SBF was stored for 20 minutes at room temperature. The reagent was aspirated using a sterile retroflush adaptor (a separate one for each SBF) and collected into a test tube. Aliquots of the sample (0.15 mL in triplicate) were placed into the wells of a 96-well tray and absorbance readings were obtained using an enzyme-linked immunosorbent assay plate reader equipped with a 595-nm filter.

Controls

For all experiments, both positive and negative controls were included. The positive control consisted of the SBF that had been inoculated with ATS containing microorganisms and that had not been subjected to any cleaning. The negative control consisted of a new SBF that had not been inoculated or subjected to any cleaning.

RESULTS

To determine the volume of the inside of the SBF shaft, it was filled with water using the retroflush lumen adaptor. A new adaptor was attached, and the water from inside the SBF shaft was aspirated and weighed. This procedure was repeated 10 times, and the average volume inside the SBF shaft was determined to be 1.07 mL. To determine the total volume of liquid that was flushed through the SBF shaft (ie, after the entire cleaning cycle), the SBF was attached to the sonic irrigator with the retroflush adaptor, then the handle was hung outside the sonic irrigator, and all fluid that emerged from the handle area was collected in a beaker, and its volume was measured. This procedure was repeated 3 times, and the average total liquid volume flushed through the SBF was determined to be 80 mL for the short (5-minute) cycle and 144 mL for the long (15-minute) cycle.

The Table summarizes the average original soil levels (ie, protein, carbohydrate, hemoglobin, and endotoxin) and the viable bioburden present in 4 SBFs used for biopsy of patients. These were new biopsy forceps that were used only once

TABLE Levels of Soil Components and Bioburden in Biopsy Forceps Used for Patient Procedures and Cleaned Manually

SBF number	Type of biopsy forceps used	Viable				
		organism count, log ₁₀ cfu per device	Protein level, µg per device	Carbohydrate level, µg per device	Hemoglobin level, µg per device	Endotoxin level, EU per device
1	Noncautery	3.63	5,058.50	82.75	7,012.50	119.90
2	Noncautery	4.81	364.75	0 ^a	252.50	306.30
3	Noncautery	6.18	751.50	305.25	355.50	2,960.00
4	Cautery	5.93	162.25	185.50	184.25	1,627.00
Mean value ± SD		5.78 ± 5.85	1,584.25 ± 2329.03	143.38 ± 131.92	1,951.19 ± 3,375.02	1,253.30 ± 1,320.84

NOTE. CFU, colony-forming units; EU, endotoxin units; SBF, single-use biopsy forceps.

^a The value "0" indicates that the soil level detected was lower than the limit of detection (see Methods).

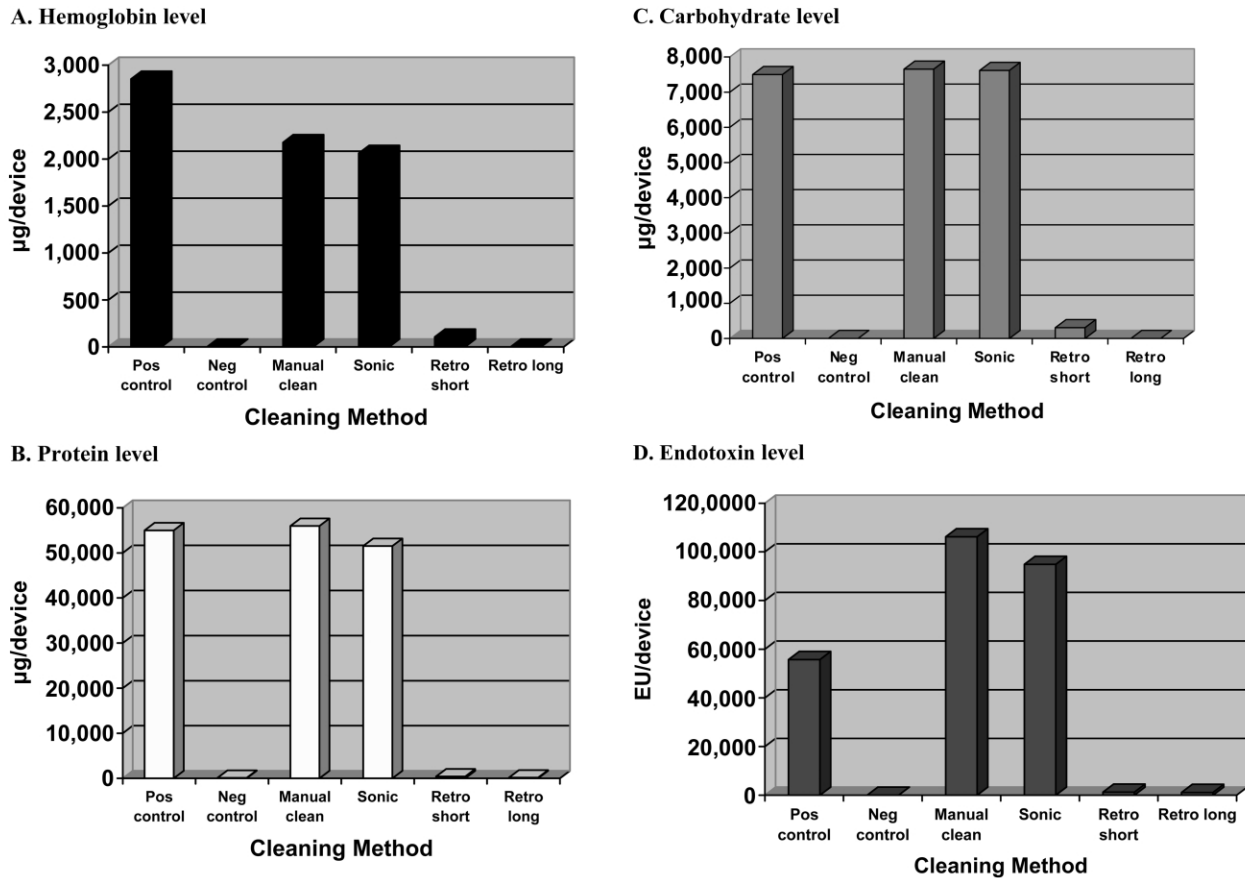


FIGURE 2. Residual soil components in single-use biopsy forceps (SBFs) after cleaning by various methods. The data presented are average values for 3 replications of the experiment. The positive control (pos control) represents the maximum amount of the soil component that can be detected by the test method used. The negative control (neg control) confirms that samples from unused SBFs do not give a positive reaction in the test assay. Retro short, automated retroflush irrigation cleaning with a 5-minute cleaning cycle; retro long, automated retroflush irrigation cleaning with a 15-minute cleaning cycle; sonic, sonication and external washing only.

during a routine gastrointestinal biopsy. The used SBF was wiped on the outside with a sterile gauze soaked in sterile water. The wiped SBF was then destructively tested to determine the levels of hemoglobin, protein, carbohydrate, endotoxin, and viable aerobic bacteria.

Figure 2 summarizes how well the various original soil components were removed by the various cleaning methods. When SBFs were cleaned using manual cleaning (no sonication) and sonication with external cleaning, there was poor removal of hemoglobin, protein, carbohydrate, and endotoxin from the inner portion of the SBF: manual cleaning removed 0%-28% of these soil components. Automated retroflush irrigation cleaning of SBFs removed 95% or more of all 4 soil components.

Figure 3 shows how effectively *E. faecalis* and *G. steartophilus* spores were removed by the various cleaning methods. Only the automated retroflush irrigation method could effect a 2 log₁₀ reduction in the concentration of microorganisms. There was little difference in the degree of reduction between the short (5-minute) and the long (15-

minute) cleaning cycles. The manual and sonication-only methods of cleaning provided less than a 1 log₁₀ reduction in the concentration of organisms.

Because destructive testing relied on elution of the original soil and viable organisms from the inner portion of the SBF lumen, we also performed a direct in situ test to assess levels of residual protein inside the lumen. The in situ method consisted of perfusing Bradford reagent into the lumens as a direct test to determine whether there was any residual protein (which is indicated by a color change). Automated retroflush irrigation cleaning was the optimal method for soil removal; however, both the destructive testing and the in situ testing showed that there were still low levels of residual protein in SBFs after cleaning with this method (Figure 4).

DISCUSSION

The results of this evaluation demonstrated that cleaning of SBFs was suboptimal by both manual and sonication-only methods. The only cleaning method that provided effective

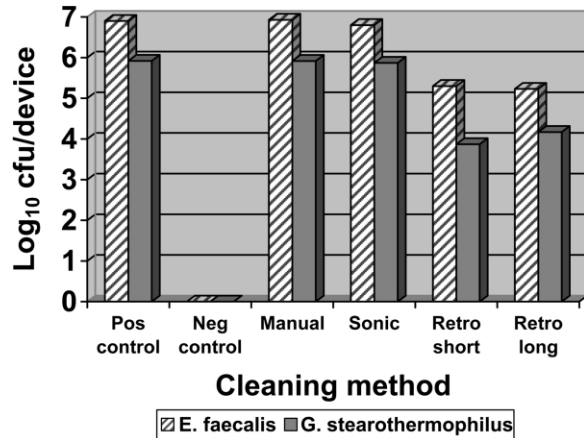


FIGURE 3. Residual bioburden of *Enterococcus faecalis* and *Geobacillus stearothermophilus* in single-use biopsy forceps (SBFs) after cleaning by various methods. The data presented are average values for 3 replications of the experiment. The positive control (pos control) represents the maximum number of viable organisms that can be detected. The negative control (neg control) confirms that unused SBFs do not have any residual viable organisms prior to use. Retro short, automated retroflush irrigation cleaning with a 5-minute cleaning cycle; retro long, automated retroflush irrigation cleaning with a 15-minute cleaning cycle; sonic, sonication and external washing only. CFU, colony-forming units.

reduction in soil and bioburden levels was cleaning with the automated, narrow-lumen irrigator that used retroflushing. Even with the retroflushing method, there was still residual protein detected by the in situ test. Although automated retroflush cleaning was more effective than manual cleaning or sonication alone, the reduction of bioburden achieved (a 2 log₁₀ reduction) was not the 3-4 log₁₀ reduction that is expected from routine cleaning. This emphasizes how difficult it is to clean such devices effectively and suggests that, with repeated use, such devices likely accumulate a build-up of original soil and bioburden over time, even when fluid is able to flush the inner channel. Facilities that are currently reprocessing SBFs using manual or automated cleaners that do not use retroflushing are providing suboptimal cleaning of SBFs.

A major reason for the suboptimal cleaning achieved by the manual and sonication-only methods is the lack of fluid flow into the shaft of the SBF. For the short and long automated cleaning cycles of retroflush irrigation, the volumes of fluid flushed through the inner shaft is 80 to 144 times the shaft volume, respectively. These values are far superior to those achieved with methods that do not actively force fluid through the shaft (eg, manual or sonication-only cleaning). The worst-case levels of viable organisms, carbohydrate, hemoglobin, and protein from SBFs used in biopsies of patients (Table 1) indicate that patient secretions do gain access to the inner channel of SBFs. The levels of original soil and bioburden recovered from SBFs inoculated with ATS by our

simulated-use protocol mimic the original soil and bioburden challenges expected in actual use.

Our data demonstrate that microbial adherence to inner lumen surfaces occurs very rapidly. After only 2 hours of contact time, it is difficult to effectively remove this bioburden, even with use of the optimal retroflush cleaning method. This emphasizes that even if the sterilization process kills the residual bioburden, there will be a gradual build-up of dead microorganisms and original soil with repeated use. It is likely that the difficulty of removing bioburden from the central lumen is related to the difficulty of achieving good fluid flow kinetics (despite good total volume of fluid flow) in the narrow lumen with retroflushing. This is likely because of the coiled wire that extends from the handle to the distal end of the SBF. This wire likely facilitates adherence of microorganisms and sequesters the organisms, making them hard to remove even when good fluid flow is achieved by retroflushing. If no fluid flow is achieved (as happens with manual or sonication-only cleaning), then there was essentially no removal of microbes or original soil. This issue would also exist for reusable biopsy forceps, but this is harder to demonstrate experimentally because the reusable biopsy forceps cannot be destructively tested.

Sphincterotomes are accessory devices that may be used in gastrointestinal endoscopic procedures. Comparison of data on cleaning of SBFs with previously published data on cleaning of sphincterotomes¹⁴ indicates that, once original soil and bioburden gains access to the inner tubular channel of SBFs, it is hard to remove. Other studies have shown that

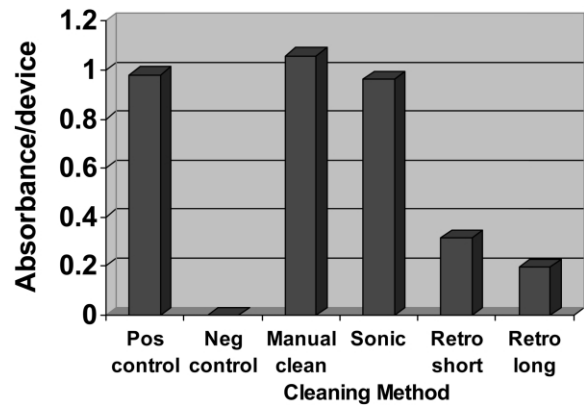


FIGURE 4. Protein levels detected in single-use biopsy forceps (SBFs) after cleaning by in situ evaluation using Bradford reagent. The data are average values for 3 replications of the experiment. The positive control (pos control) indicates that the maximum protein level detected has an absorbance of 1. The negative control (neg control) demonstrated that there was no reaction triggered when Bradford reagent was instilled into the channel of an SBF that had not been soiled. Retro short, automated retroflush irrigation cleaning with a 5-minute cleaning cycle; retro long, automated retroflush irrigation cleaning with a 15-minute cleaning cycle; sonic, sonication and external washing only.

there are similar difficulties in removing original soil from medical devices when physical constraints exist (eg, lumens or serrated edges).^{11,16-20} Alfa et al.¹⁴ have shown that, despite the 3 narrow lumens (1 containing a wire) that run the length of sphincterotomes, they could be adequately cleaned, after 24 hours of drying, with automated retroflush irrigation; no protein was detected by in situ Bradford testing or destructive quantitative protein testing. Even the bioburden concentrations in sphincterotomes were reduced by 3-4 log₁₀ with the retroflush method. Unlike sphincterotomes, the SBFs tested in this study had low levels of residual hemoglobin and carbohydrate, as well as high residual bioburden concentration, when the devices were dried for 2 hours then cleaned with retroflushing in an automated narrow-lumen cleaner. Even a more prolonged washing cycle (15 minutes instead of 5 minutes) did not provide complete removal of protein or reasonable bioburden reduction. How the data from this study relate to third-party reprocessing protocols is not known, because such protocols involve proprietary information. However, during the review process, the US Food and Drug Administration requires third-party reprocessors to provide data to support their claims about both cleaning and sterilization.

This study was aimed at assessing methods for cleaning SBFs and did not assess whether sterility could be achieved by subsequent ethylene oxide sterilization. The amount of original soil that would lead to low-temperature sterilization failure is unknown. However, our data raises questions about the ability to reprocess SBFs, because basic cleaning cannot be reliably achieved. As shown by Marshburn et al.,²¹ if hardy organisms remain, both ethylene oxide and steam can fail to sterilize medical devices reliably.

In conclusion, cleaning of the inner tubular cavity of soiled SBFs was only achieved using the automated retroflush cleaning method. Although original soil was effectively removed by the retroflush method, this method did not achieve the 3-4 log₁₀ reduction in bioburden that is expected from routine cleaning. Comparison of our data on SBFs with published data on cleaning of sphincterotomes suggests that soil and bioburden build-up over repeated uses is likely to occur at a faster rate for SBFs than for sphincterotomes. On the basis of the data in this study, we recommend that extended reuse of SBFs would not be optimal, because adequate cleaning could not be achieved using the equipment and methods currently available to healthcare facilities.

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None of the authors have any proprietary interest in the medical devices studied or the data presented in this article.

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