Turbulent Fluid Flow is a novel closed-system sample extraction method for flexible endoscope channels of various inner diameters

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Abstract:

Overview: Effective sample extraction from endoscope channels is crucial for monitoring manual cleaning adequacy as well as for ensuring optimal sensitivity for culture after disinfection. The objective of this study was to compare the efficacy of Turbulent Fluid Flow (TFF) to Flush (F) or Flush-Brush-Flush (FBF) methods.

Materials & Methods: *Pseudomonas aeruginosa* and *Enterococcus faecalis* in artificial test soil-2015 (ATS2015) were used as bacterial markers while protein and carbohydrate were the organic markers for biofilm formed inside 3.2-mm and 1.37-mm polytetrafluoroethylene (PTFE) channels. TFF was generated using compressed air and sterile water to provide friction for sample extraction. Extraction for biofilm coated PTFE channels as well as for colonoscope channels perfused with ATS2015 containing 10^8 CFU/mL *P. aeruginosa, E. faecalis* and *Candida albicans* was determined using TFF compared to FBF and F.

Results: The extraction ratio for *P. aeruginosa* and *E. faecalis* from biofilm extracted by TFF compared to the positive control was significantly better than F for 1.37-mm channels ($\geq$ 0.94 for both bacteria by TFF versus 0.69 to 0.72 by F for *P. aeruginosa* and *E. faecalis*, respectively) but not significantly different between TFF and FBF for 3.2-mm channels. F was also ineffective for extraction of protein and carbohydrate from 1.37-mm channels. Extraction efficacy by TFF from inoculated colonoscope channels was >98% for all test markers.

Conclusions: The novel TFF method for extraction of samples from colonoscope channels is a more effective method than the existing FBF and F methods.

Introduction:

Outbreaks of multi-drug resistant organisms (MDRO) due to contaminated flexible endoscopes have occurred world-wide (Murray 2016, Higa et al. 2016, Aumeran et al. 2012, Rauwers et al. 2017, Epstein et al. 2014, Kola et al. 2015, Verfaillie et al. 2015). This has focused attention on the use of culture methods to detect endoscope channel contaminants that are organisms of concern (i.e. organisms associated with infectious outbreaks transmitted from contaminated endoscopes) (Cattoir et al. 2017, Alfa et al. 2017a, Gazdik et al. 2016, Beilenhoff et al. 2006, US-FDA 2015, FDA-CDC-ASM guideline 2018). There are a multitude of methods that have been reported for extracting endoscope channel samples including flushing various types of extraction fluids (e.g. sterile reverse osmosis (sRO) water, neutralizing pharmacopeia diluent (NPD), buffer solutions, Tween containing fluids, various broth media) combined with brushing of some channels to provide friction (Beilenhoff et al. 2006, Alfa et al. 2017a, Gazdik et al. 2016, FDA-CDC-ASM guideline 2018, Systchenko et al. 2000, Rauwers et al. 2017). Friction has been shown to be a critical factor to ensure optimal sample extraction from PTFE channels (Alfa et al. 2017a) and has traditionally been achieved using a channel bristle brush or pull-through channel cleaners with a flush-brush-flush extraction process (Brock et al. 2015, Alfa et al. 2017b, FDA-CDC-ASM guideline 2018, Rauwers et al. 2018). These bristle brushes and pull-through cleaners were originally designed to be used during the manual channel cleaning process. However, there are narrow endoscope channels for which there are no available channel brushes (e.g. air-water channels, auxiliary water channels and some ureteroscope channels). In addition to the variability of extraction fluids used for endoscope channel sample collection in the published literature, there is also variability in the recommendations for using channel

brushes to provide friction (AS/NZS 4187 2014, Devereaux et al. 2019, Beilenhoff et al. 2006, Systchenko et al. 2000, ANSI/AAMI ST91 2015, FDA-CDC-ASM guideline 2018).

The interim duodenoscope channel extraction method for culture that was employed in the Epstein et al. (2014) outbreak investigation by the CDC has been replaced with the standardized duodenoscope sample collection protocol released as the FDA-CDC-ASM guideline in 2018. This method uses sRO (or sterile deionized) water for the extraction fluid along with sterile channel bristle brushes for a FBF sample extraction from the instrument channel of duodenoscopes. The method also recommends Dey-Engley broth as a neutralizer that is added in a 1:1 ratio to the channel sample immediately after collection. The guideline also requires concentration of the sample for culture (e.g. filtration or centrifugation) such that the entire sample is inoculated on blood agar media. This method has been validated by endoscope manufacturers including Olympus, Pentax and FujiFilm to provide between 65% to 100% extraction efficacy for a duodenoscope instrument channel and lever recess. Despite this excellent advancement for duodenoscope sample collection, there is no validated method to provide friction for sample collection from narrow channels such as the air-water channel or auxiliary channels of duodenoscopes or for other types of flexible endoscopes (e.g. colonoscopes, gastroscopes, bronchoscopes). Furthermore, the use of a channel bristle brush to provide friction during sample collection of the instrument channel creates a risk for introducing environmental contaminants during sample collection as the sterile brush shaft can be difficult to control and may inadvertently touch external parts of the endoscope or environmental surfaces. As such there is a need to further improve sample extraction from flexible endoscope channels that will provide friction to optimize sample extraction and reduce the risk of environmental

contaminants during sample collection. This is especially important for channels such as the AW and AUX channels that cannot be brushed as they are too narrow to pass a long brush down the entire length (e.g. many of the models with an AW channel bifurcate into two channels near the distal end and the brush cannot reach both channels after the bifurcation).

One approach for removing adherent organic and microbial residues from the inner channel surface is turbulent fluid flow (TFF) (Labib et al. 2011). This technology provides droplet flow driven by a high-velocity turbulent air stream to achieve high shear stress at the surface of a narrow channel. The authors reported that this TFF technology may be ideal for cleaning of narrow channels in flexible endoscopes. However, there has been no assessment of this technology for endoscope channel sample collection.

The objective of this study was to evaluate the novel TFF technology as a means of providing optimal friction in a "closed system" for extraction of biofilm formed inside PTFE channels and extraction of inoculated colonoscope channels.

Materials and Methods:

Microbial strains and Culture methods:

Three microbial strains were purchased from the American Type Culture Collection (ATCC, Manassas, VA): *Enterococcus faecalis* (ATCC 29212), representative of a Gram positive bacteria that has been associated with contaminated endoscopes, *Pseudomonas aeruginosa* (ATCC 27853), representative of a Gram negative bacteria that has been associated with contaminated endoscopes and *Candida albicans* (ATCC 14053), representative of a yeast that has been associated with contaminated endoscopes. Before experiments, *E. faecalis* and *P. aeruginosa* were sub-cultured on blood agar consisting of tryptic soy agar containing 5% (v/v) sheep blood (Lampire, Pipersville, PA) and *C. albicans* (CA) was sub-cultured on Sabouraud dextrose agar from frozen stocks and incubated aerobically at 35 °C for 24 hrs. All microbial strains were sub-cultured three times before use. Extracted endoscope channel samples were serially diluted in phosphate buffered saline (PBS) to 10^{-8} and 100 µL from each dilution was plated onto CHROMagar orientation media (BD, Sparks, MD).

Artificial Test Soil-2015 (ATS2015)

Artificial Test Soil-2015 (Healthmark Industries, Fraser, MI) was rehydrated as per the manufacturer's instructions for use (MIFU) and supplemented to a final concentration of 20% sheep blood (Lampire, Pipersville, PA). This ATS2015 containing 20% blood has been shown to mimic the secretions from patient-used flexible endoscopes (Alfa and Olson 2016) so is an appropriate test soil for developing biofilm and inoculation of the colonoscope for simulated-use testing.

Sample Neutralizer:

The double-strength (2X) neutralizer used was that described by Pineau and De Philippe (2013) (Pineau neutralizer) and consisted of Tween 80 (Sigma, St Louis, MO) 3% (v/v), lecithin (Sigma) 0.3% (w/v), L-histidine (Sigma) 0.1% (w/v), and sodium thiosulfate (Sigma) 0.5% (w/v). Sterile Pineau neutralizer was added immediately after sample extraction in equal volume to all test aliquots extracted from colonoscope channels that were used for culture to facilitate growth of microbes that have been potentially damaged by the reprocessing process (as outlined in the FDA-CDC-ASM guideline (2018).

Traditional biofilm formation in 3.2-mm and 1.37-mm Polytetrafluoroethylene (PTFE) channels:

Both 3.2-mm inner diameter PTFE tubing (catalogue # 5239K11, McMaster-Carr, Robbinsville, NJ) and 1.37-mm inner diameter tubing (catalogue # 137003, Endoscopy Development Company, Maryland Heights, MO) were the new endoscope channels used for formation of traditional biofilm. The ATS2015 was inoculated with *E. faecalis* and *P. aeruginosa* each at 10^8 CFU/mL. The ATS2015-bacterial suspension was perfused through a sterile PTFE channel and then connected to form a closed circuit so that the inoculum was continuously circulated through the PTFE channel using a peristaltic pump (MasterFlex C/L Model 77122-14, Cole-Parmer, Barrington, IL) at a flow rate of 72 mL/hr at room temperature. After overnight circulation, the suspension was drained and the channel was rinsed three times with sRO water, and then continuously perfused overnight with a 1:10 dilution of ATS2015 containing *E. faecalis* and *P. aeruginosa* each at 10^5 CFU/mL. For each of three following mornings the draining, rinsing, and soiling of the channel was repeated exactly as per the second day. On the last day, the channel

was rinsed with sRO water as per previous days. For storage, the biofilm containing PTFE channel was filled with sRO water and stored at room temperature to prevent drying. This formation of biofilm within PTFE channels represents a "worst-case" challenge.

Colonoscope testing:

An Olympus CF Type H180L (Olympus-180) colonoscope was used. The colonoscope was reprocessed following the MIFU with high level disinfection achieved using Peracetic acid (4.5%), Angelini Pharma Inc (Gaithersburg, MD) followed by tap water rinsing. The reprocessed colonoscope was thoroughly air dried by flushing air through the channels prior to storage. The benchmarks for adequate colonoscope channel cleaning for protein and carbohydrate were < 6.4 $\mu g/cm^2$ and < 1.2 $\mu g/cm^2$, respectively (Alfa et al. 1999).

For inoculation of the colonoscope a suspension containing *E. faecalis*, *P. aeruginosa*, and *C. albicans* at 10^8 CFU/mL in ATS2015 (ATS-EPC) was prepared. The colonoscope was laid out on new absorbent pad (Shield Line, Hackensack, NJ) on a table and the distal end was placed on sterile gauze. Sterile connectors and plugs were attached to the endoscope. To soil the suction-biopsy (SB) channel, the sterile biopsy port plug was removed and a syringe containing ATS-EPC was used to flush the inoculum slowly through the entire SB channel with the distal end raised up until fluid just emerged from the distal end. To soil the Air-Water (AW) channel, a syringe containing the ATS-EPC was used to slowly flush the inoculum through the channels until fluid just emerged from the distal end. To soil the Auxiliary water (AUX) channel, a syringe containing ATS-EPC was flushed slowly through the AUX channel until soil just emerged from the distal end. After soiling, the excess fluid was drained by flushing each

169 inoculated channel with 60 cc air three times. The inoculated channels were then allowed to dry
170 at room temperature for two hours.
171
172 **Turbulent Fluid Flow (TFF) sample extraction from channels:**
173 ***PTFE channels containing traditional biofilm (PTFE-TBF):***
174 A 30.5 cm length of PTFE-TBF was cut using a sterile scalpel. A channel extraction apparatus
175 (CEA) was created by connecting the PTFE-TBF segment between two flanking segments of
176 sterile PTFE tubing that were 76.25 cm in length and had same ID (internal diameter) as the test
177 section to make a total length of 183 cm using connectors (Figure 1). All connectors and the
178 TFF water pump head, and bottle cap manifold were steam sterilized prior to use. A sterile
179 sample collection bottle was attached to the sterile bottle cap manifold for channel sample
180 collection. The two HEPA filters and one end of the CEA containing the test section were
181 connected to the manifold. The other end of the CEA was connected to the TFF mixing chamber.
182 The compressor was started and the air pressure was adjusted to 28 psi. The pump (FMI
183 Q1SAN) setting and the controller (FMI V200) setting were adjusted accordingly for different
184 PTFE tubing ID such as 3.2-mm and 1.37-mm. After the pump was turned on, the air valve was
185 opened to generate TFF then 100 mL of sRO water was used for each channel extraction. Once
186 the sRO water was finished, the pump was stopped and the air valve was closed. A 3-mL aliquot
187 of the sample in the collection bottle was stored at -20 ^{o}C for chemistry testing and then the
188 remaining sample was used for viable count. A portion of the extracted sample was serially
189 diluted and 0.1 mL of each dilution was spread over the surface of a CHROMagar plate and
190 incubated aerobically at 35 ^{o}C for 24 hours. The remainder of the sample was concentrated
191 using a sterile filtration apparatus (MicroFunnel, Pall Corporation, Ann Arbor, MI) and the filter

was aseptically removed and transferred onto a CHROMagar plate. The inoculated agar medium was incubated aerobically at 35 ^{o}C for 72 hours and the CFU (colony forming unit) was determined.

Turbulent fluid flow sample extraction from Colonoscope channels:

Sterilized connectors and plugs were attached to the appropriate outlets of the SB, AW and AUX channels of an Olympus-180 colonoscope (Figure 2). The distal end of the endoscope was attached to a sterile manifold that provided HEPA venting of air and collection of the fluid in a sterile collection container (TFF endoscope sample collection as shown in Figure 2). The compressor was started and the air pressure was adjusted to 28 psi. The pump (FMI Q1SAN) setting and the controller (FMI V200) setting were adjusted appropriately for each of the SB, AW or AUX channel. The flow rate was 22 mL/min for SB, 18 mL/min for AW, and 14 mL/min for AUX channel. After the pump was turned on, the air valve was opened to generate TFF. Sample extraction was achieved using 100 mL of sRO water for each harvesting. Once the sRO water was finished, the pump was stopped and the air valve was closed. For harvesting a specific channel, the channels not in use were clamped. The extracted sample was collected in a sterile container. A 2-mL aliquot of the extracted sample was kept frozen for chemistry testing and the remaining sample had 2X Pineau neutralizer added and was used for serial dilution and viable count (as described previously). After extraction of one channel, the distal end was dipped in sRO water and then wiped with an alcohol swab and air dried prior to collecting the next channel sample.

Quantitation of viable bacteria, protein and carbohydrate:

Unless specified otherwise, a 3-mL aliquot of the 100-mL TFF sample was removed to a sterile container and frozen for protein and carbohydrate testing. The remaining extracted sample had an equal volume of 2X Pineau neutralizer added. For positive controls the neutralized sample was serially diluted 1:10 and 0.1 mL of each dilution inoculated onto CHROMagar medium. For negative controls and samples expected to have low CFU, the entire neutralized sample was concentrated by filtration as recommended in the FDA-CDC-ASM guideline (2018). Results were reported as CFU/cm^2 and the limit of detection was 10 CFU/mL for unconcentrated enumeration and 1 CFU/97 mL for concentrated enumeration. Protein was assessed using the QuantiPro BCA assay (Sigma, St Louis, MO), which included a bovine serum albumin protein standard. This quantitative assay is based on bicinchoninic acid and the limit of detection was 0.5 µg/mL. The carbohydrate assay described by Liu et al. (1994) was used and the limit of detection was 10 µg/mL. Protein and carbohydrate assays were performed following the manufacturers' instructions and results were converted to micrograms per square centimeter (µg/cm^2).

Calculation of biofilm extraction ratio from PTFE channels (3.2-mm and 1.37-mm):

Reliable quantitation of microbial levels within biofilm is difficult. Waller et al. (2018) demonstrated that sonication optimizes biofilm detachment for determining CFU. In order to compare the efficacy of FBF and F sample extraction to TFF extraction, destructive testing combined with sonication and vortex mixing was used as the positive control for viable counts (i.e. maximum level of viable cells that could be extracted). Similar to Aumeran et al. (2012)'s approach, the viable count for a defined length of PTFE channel was expressed as Log_{10} CFU/cm^2 and the ratio of this viable count was compared to that of the positive control (i.e.

extraction ratio). The higher the extraction ratio the more effective the sample extraction method.

Calculation of extraction efficacy from flexible endoscope channels:

Destructive testing is not possible for endoscopes so an alternative method to determine extraction efficacy is needed. For endoscope channels that are inoculated with a test soil containing viable bacteria, the extraction efficacy for each method evaluated was determined using repeated rounds of extraction (i.e. exhaustive extraction) that is indicated in the FDA 2015 Guide to Manufacturers (2015). Three repeat rounds of extraction from endoscope channels were each collected separately. The CFU/cm^2, as well as μg/cm^2 for both protein and carbohydrate were calculated for each round of extracted material. The percentage efficiency of the initial round of extraction was calculated as: C1/(C1+C2+C3) x 100 where C1 is the CFU/cm^2 for the first round of extraction, C2 is the CFU/cm^2 for the second round of extraction and C3 is the CFU/cm^2 for the third round of extraction (C1+C2+C3 represents the maximum extractable amount of the CFU test marker). This same process was also used to determine the percentage extraction efficacy for μg/cm^2 of protein and carbohydrate test markers from each round of extraction.

Overview of experimental testing:

PTFE channels: The exraction efficacy of FBF (for 3.2-mm channels), F (for 1.37-mm channels) and TFF (for both 3.2-mm and 1.37-mm channels) were compared to destructive testing for microbes as well as protein and carbohydrate.

Colonoscope channels: The extraction efficacy of FBF (for SB channel) and F (for AW and AUX channels) were compared to TFF (for SB, AUX and AW channels) for microbes as well as protein and carbohydrate.

All experiments were performed in triplicate unless otherwise stated.

Statistical analysis:

The student t-test (2 tailed) was used to analyze the Log_{10} CFU/cm^2 (or μg/cm^2 for organic residuals) data for biofilm testing and to analyze the % extraction efficacy based on CFU/cm^2 (or μg/cm^2 for organic residuals) for the endoscope inoculation testing.

Results:

The initial testing of extraction efficacy was done using PTFE channels containing traditional biofilm formed as described by Alfa et al (2017b). Destructive testing (Alfa et al. 2017a) of biofilm coated PTFE channels was used as the positive control (POS). The biofilm extraction efficacy of TFF, FBF and F for bacterial and organic residues (protein and carbohydrate) from 3.2-mm PTFE channels as well as from 1.37-mm PTFE channels was compared to the POS control (Table 1). When performing simulated-use testing with biofilm coated 3.2-mm PTFE channels, the extraction of *E. faecalis, P. aeruginosa*, protein and carbohydrate was not significantly different for TFF versus FBF. Whereas, for 1.37-mm biofilm coated PTFE channels TFF had significantly better extraction ($p < 0.001$) for *E. faecalis, P. aeruginosa*, and protein and was trending to significance ($p = 0.062$) for carbohydrate.

Compared to the POS the extraction ratio for *E. faecalis* from 3.2-mm biofilm coated channels was 1.0 and 0.92 for TFF and FBF, respectively. The extraction ratio from 1.37-mm biofilm

286 coated channels was 1.0 for TFF but only 0.72 for F. Similarly, for *P. aeruginosa* the extraction
287 ratio was similar for TFF and FBF in 3.2-mm biofilm coated channels (0.97 and 0.98,
288 respectively) but for 1.37-mm biofilm coated channels the ratio was 0.94 and 0.69 for TFF and F
289 respectively. The poor extraction ratio ($p < 0.001$) for F compared to the POS was also apparent
290 for protein and carbohydrate in the 1.37-mm channels (Table 1).
291
292 For sample extraction from endoscope channels destructive testing is not possible, so TFF
293 extraction was compared to FBF and F extraction methods as outlined in the FDA-CDC-ASM
294 guideline for duodenoscope channel sample collection (2018). The test markers included; CFU,
295 protein and carbohydrate. The results of this comparison are shown in Tables 2 and 3.
296 The TFF extraction efficacy (i.e. first round of extraction) for microorganisms was > 98% for all
297 colonoscope channels tested, whereas the FBF and F sample collection method could not achieve
298 this level of extraction efficacy for any of the channels tested (Table 3 shows that the microbe
299 extraction efficacy for F and FBF ranged from 83.6% to 95.8%). Overall, the TFF extraction
300 efficacy from inoculated colonoscope channels was significantly better than FBF or F sample
301 collection for microbial and organic markers from the SB and AUX channels with 8/15 test
302 parameters being significantly better for TFF extraction and 0/16 test parameters being
303 significantly better for FBF or F sample extraction (Tables 2 and 3). For the AW channel the
304 extraction efficacy of TFF versus F was not significantly different for any of the microbial or
305 organic markers. This is likely due to the higher variability of the FBF and F sample collection
306 methods (i.e. higher standard deviation).
307

For organic markers, the TFF extraction method was > 99% effective for all channels tested whereas none of the FBF or F methods achieved this level of extraction efficiency (Table 3 demonstrates that the extraction efficacy for FBF and F ranged from 89.1% to 98.8%**)**. The high variability in extraction efficacy was also apparent for protein and carbohydrate using the F extraction method for the AW channels of the colonoscope (Table 3) compared to the TFF extraction method (Table 2).

The average of negative controls for 3.2-mm PTFE channels showed no viable organisms, protein < 2.1 $\mu g/cm^2$ and carbohydrate < 0.45 $\mu g/cm^2$. The results for the 1.37-mm PTFE channels were similar to the 3.2-mm PTFE channels except that the carbohydrate levels were < 5.2 $\mu g/cm^2$. The negative controls for the colonoscope for TFF testing showed on average < 0.051 CFU/cm^2 of *E. faecalis, P. aeruginosa* or *C. albicans* (Table 4) and < 0.3 $\mu g/cm^2$ for protein or carbohydrate from the SB, AW and AUX channels. For the FBF and F sample collections from the colonoscope the negative control results were similar to those for the TFF testing (Table 4) except for carbohydrate that on average was < 0.6 $\mu g/cm^2$. The negative controls were taken after full reprocessing and storage demonstrating that the detection of viable organisms prior to experimental testing was rare and that the average protein and carbohydrate residuals were within the benchmarks for adequately cleaned channels.

Discussion:

Our data demonstrated, not unexpectedly, that destructive testing was an optimal positive control in terms of extraction efficacy of high levels of viable bacteria and organic markers from biofilm within narrow lumen channels (i.e., simulated-use testing using a worst-case surrogate channel

model). Our data support other studies (Waller et al. 2018, Johani et al. 2018, Aumeran et al. 2012) that used destructive testing and sonication to optimize biofilm detachment. Waller et al. (2018) reported that 3.4 to 6.1 x 10^6 CFU/mL were extracted from biofilm with sonication whereas only 1 x 10^2 CFU/mL were extracted without sonication. Our results for destructive testing are similar to Cattoir et al. (2017)'s data where destructive testing was used for their positive controls. They tested *P. aeruginosa* biofilm coated PTFE channels and compared the extraction efficacy of 10 mL saline flush, 10 mL neutralizer (NPD) flush, 10 mL saline with FBF using a bristle brush and 10 mL saline with a pull-through device. They used destructive testing for positive controls and reported that the extraction efficacies of the four sample collection methods they studied ranged from 44% to 59% (Cattoir et al. 2017). Aumeran et al. (2012) had also used *P.aeruginosa* biofilm in channels to assess extraction efficacy by flushing using either water, saline or Letheen broth. The destructive testing positive control showed levels of *P. aeruginosa* in their biofilm (i.e. 10^7 to 10^8 CFU/cm^2) similar to the CFU/cm^2 in the biofilm used for our evaluation. Aumeran et al. (2012) found that flushing with water and Letheen broth had extraction ratios of 0.84 and 0.93, respectively. Our testing evaluated different methods of generating friction for sample extraction. It confirmed that higher extraction ratios could be achieved in 3.2-mm PTFE channels (0.97 to 1.00 for TFF and 0.92 to 0.98 for FBF) compared to when no friction was used in 1.37-mm PTFE channels (0.69 to 0.72 extraction ratio for F extraction). Our data support Aumeran et al. (2012)'s approach of assessing extraction efficacy of viable bacteria from biofilm using the concept of "extraction ratio" of the test method compared to an appropriate positive control.

353 The TFF method of creating friction to extract channel samples provides an alternative to the use
354 of bristle brushes or a pull-through device used by Cattoir et al (2017). Unlike the other methods
355 of creating friction, TFF can be used in a closed sample collection process that does not create
356 aerosols and reduces the risk of environmental contamination of the sample. Our data and that of
357 Cattoir et al. (2017) further support the study by Alfa et al. (2017a) where friction was shown to
358 be a critical factor in sample extraction from PTFE channels coated with build-up-biofilm which
359 is more difficult to remove than traditional biofilm.
360
361 Our current study demonstrated that extraction of organic residuals such as protein and
362 carbohydrate from biofilm coated PTFE channels was also challenging. Although many studies
363 have been done to assess the ability of enzymatic and non-enzymatic detergents to remove
364 organic material in biofilms, this aspect is not well studied in terms of extraction of endoscope
365 channel samples to determine the efficacy of manual cleaning. The extraction efficacy of TFF
366 was not significantly different from that of FBF for 3.2-mm PTFE channels for protein or
367 carbohydrate but for 1.37-mm PTFE channels there was significantly more protein extracted
368 with TFF compared to F (TFF vs F was also trending to significance with carbohydrate
369 extraction). The authors are not aware of other published studies that evaluated extraction of
370 organics from biofilm within PTFE channels in terms of monitoring extraction efficacy. Because
371 TFF sample extraction is achieved using sterile RO water (i.e. no surfactants or other additives)
372 there would be no interference with ATP assays or with quantitative assays for protein or
373 carbohydrate.
374

375 Since destructive testing is not feasible for reusable medical devices, the extraction of residuals
376 from narrow channels of such devices has been focused on the use of F and FBF methods for
377 cleaning validation (Alfa et al. 2017a, Visrodia et al. 2017, Ma et al. 2018, Pineau and De
378 Philippe 2013) and culture (e.g. Cattoir et al. 2017, FDA-CDC-ASM guideline 2018). Indeed,
379 Cattoir et al (2017)'s review of various National guidelines for endoscope sample collection
380 indicated that fluid volumes ranging from 1 mL to 200 mL were recommended for F or FBF
381 sample collection. The only published alternative approach is pump-assisted flushing using 50
382 mL of neutralizer fluid for extraction from endoscopes (Ji et al. 2018). This method was
383 significantly better than manual flushing for patient-used flexible endoscopes (in terms of CFU
384 levels detected) but no simulated-use data comparing the extraction efficacy of the pump-assisted
385 method to the manual method was provided. Gazdik et al. (2016) also reported that in addition
386 to flushing the instrument channel with fluid, the use of a flocked swab instead of the larger
387 cleaning brush recommended by the CDC interim protocol, improved the recovery of
388 *Escherichia coli* (46%), *P. aeruginosa* (80%), and *E. faecalis* (67%) from the lever recess of
389 duodenoscopes. The need to standardize and validate the extraction methods used for sample
390 collection from flexible endoscopes has been recognized (Rauwers et al. 2017, Cattoir et al.
391 2017, Gazdik et al. 2016) but many of the published studies do not provide extraction efficacy
392 data for the sample collection method they used (Olafsdottir et al. 2018, Rauwers et al. 2017,
393 Shin and Kim 2015, Ji et al. 2018, Ma et al. 2018). The recently released FDA-CDC-ASM
394 guideline (2018) sample collection protocol for duodenoscopes is one of the few studies where
395 the three main endoscope manufacturers validated the extraction efficacy of the culture protocol.
396 The manufacturer testing using the FBF method for duodenoscopes in the FDA-CDC-ASM
397 guideline (2018) method achieved extraction efficacy of 65% to 100%.

Our data evaluating non-destructive sample extraction for inoculated endoscope channels are the first to document that overall TFF extraction is superior to F only and FBF extraction methods for extraction of both microbial and organic residuals. Extraction efficacy was > 98% for all channels for both *P. aeruginosa* and *E. faecalis* and for protein and carbohydrate the TFF extraction efficacy was > 99% for all channels tested. This TFF extraction was superior to the 89.5% extraction using FBF from intubation endoscopes (Alfa et al. 2016) perfused with ATS2015 containing high bacterial levels. Unlike extraction from biofilm-coated PTFE channels, the extraction of samples from colonoscope channels perfused with ATS2015 containing high microbial levels mimics clinical material suctioned through endoscopes (Alfa et al. 2016). This study demonstrated that TFF can provide optimal sample extraction for patient-used colonoscopes for cleaning verification testing as well as for culture testing after HLD (High Level Disinfection; with or without storage). The testing performed in this study facilitates the harmonization of the TFF sample collection with the FDA-CDC-ASM guideline (2018) approach for culture to detect contamination of endoscope channels (i.e. sample extraction from the BP to distal end). However, further testing is needed to assess TFF extraction from the lever and lever recess of duodenoscopes. Endoscope manufacturers validated the FDA-CDC-ASM guideline (2018) FBF method of sample extraction from the duodenoscope instrument channel (BP to distal end) and lever recess as between 65 to 100% effective. Our data demonstrated that TFF may be a more reproducible extraction method for achieving > 98% extraction efficacy from all endoscope channels irrespective of the inner diameter. Our simulated-use biofilm extraction data indicates that if biofilm was present in endoscope channels, the TFF extraction method would provide efficient extraction of this type of more challenging residual.

Furthermore, all TFF sample collection can be performed by one person. This aspect could facilitate the ability of busy endoscopy clinics to initiate sample collection for cleaning validation as well as for post-HLD culture testing of endoscope contamination.

Limitations of this study include that only colonoscope channels from one manufacturer were evaluated and that further studies are needed to optimize the TFF channel extraction for other types of levered and non-levered flexible endoscopes from various manufacturers.

In summary, the key findings for the TFF extraction from flexible endoscope channels includes; optimal friction is provided using TFF which can be achieved in all channels even those that currently do not get brushed and it is a closed system thereby reducing the risk of extraneous contamination associated with the FBF protocol.

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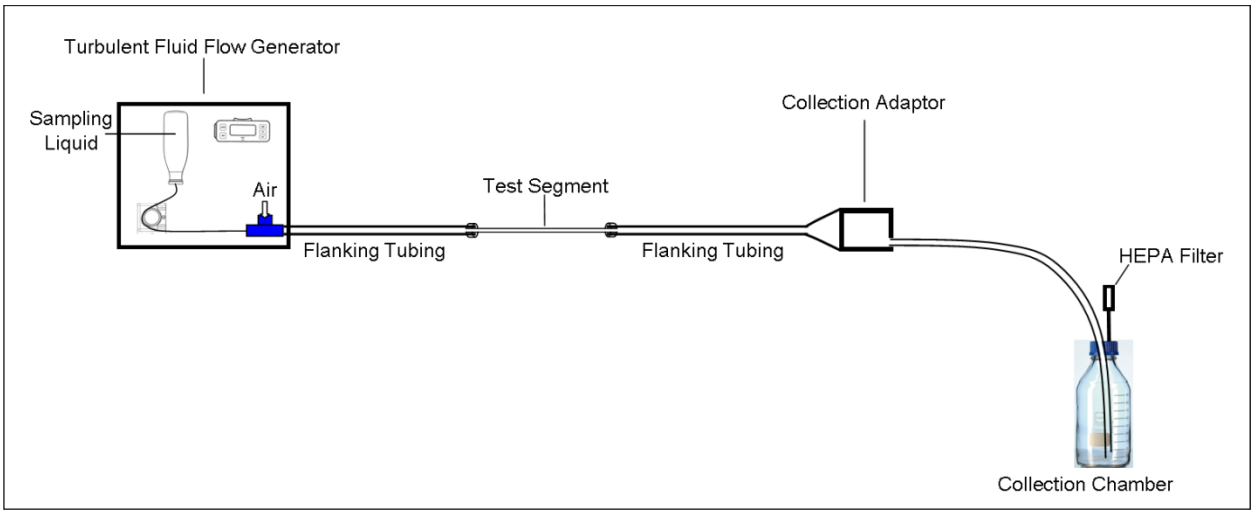
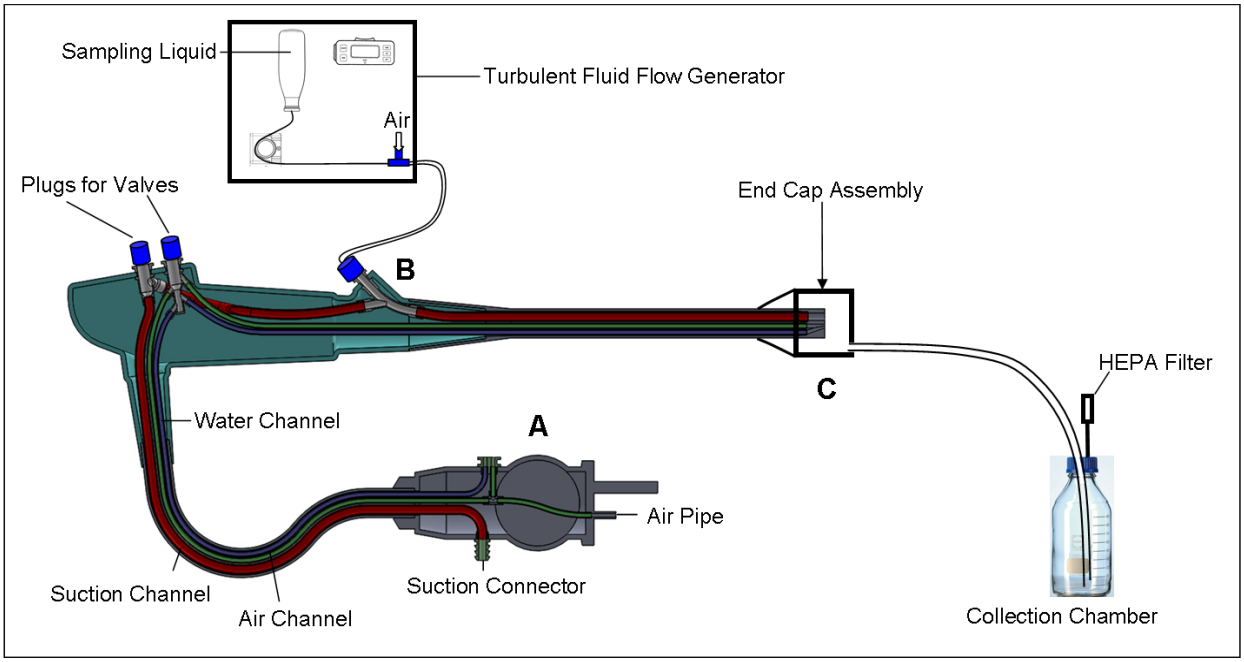
Figure 1 Turbulent fluid flow generation device connected to biofilm-coated PTFE test segment



The TFF connection setup for CEA where the test segment of biofilm coated PTFE channel (either 3.2 mm or 1.37 mm inner diameter) is inserted between sterile flanking tubing to provide a total length similar to a colonoscope.

Figure 2 Turbulent fluid flow generation device connected to inoculated colonoscope



The TFF connection setup for sample collection from an inoculated colonoscope from the Biopsy port (B) to the distal end (C). For the Air/Water and Auxiliary water channels the TFF was delivered from the umbilical end (A) to the distal end (C) with a plug in the handle area. The Auxiliary water channel from umbilical to distal end is not shown in the above diagram.

Table 1: Extraction of Microbial and Organic markers by Turbulent Fluid Flow, Flush-brush-flush, and Flush sample collection compared to destructive extraction of traditional biofilm in 3.2-mm and 1.37-mm PTFE channels.

	3.2 mm PTFE Channel:			1.37 mm PTFE Channel:		
	TFF[1]	FBF[2]	POS[3]	TFF[1]	F[4]	POS[3]
E. faecalis	Log_{10} CFU/cm²					
Experiment 1	6.23	5.59	5.90	6.22	4.04	6.09
Experiment 2	6.08	5.49	6.19	6.02	4.51	6.09
Experiment 3	5.42	5.09	5.45	5.92	4.24	5.64
Average (STD[5]):	5.91 (0.43)	5.39 (0.27)	5.85 (0.37)	6.05[6] (0.15)	4.26 (0.23)	5.94 (0.26)
P. aeruginosa	Log_{10} CFU/cm²					
Experiment 1	7.24	7.17	7.29	7.44	5.28	7.90
Experiment 2	7.06	7.26	7.40	7.36	5.77	7.81
Experiment 3	7.09	7.12	7.34	7.57	5.47	8.15
Average (STD[5]):	7.13 (0.10)	7.18 (0.07)	7.34 (0.05)	7.46[6] (0.10)	5.51 (0.25)	7.95 (0.18)
Protein	µg/cm²					
Experiment 1	8.16	10.09	16.26	12.07	0.00	26.06
Experiment 2	7.02	7.92	13.41	11.44	0.00	28.15
Experiment 3	6.39	5.93	13.41	10.77	0.11	27.54
Average (STD[5]):	7.19 (0.90)	7.98 (2.08)	14.36 (1.65)	11.43[6] (0.65)	0.04 (0.06)	27.25 (1.07)

Carbohydrate	**µg/cm²**					
Experiment 1	8.93	16.37	8.83	6.20	0.85	13.55
Experiment 2	9.45	13.47	8.53	27.30	1.91	14.33
Experiment 3	15.90	5.18	8.05	18.87	2.12	16.60
Average STD[5]:	11.43 (3.88)	11.67 (5.81)	8.47 (0.39)	17.46[7] (10.62)	1.62 (0.68)	14.83 (1.58)

The extraction efficacy ratio is calculated as $Log_{10}CFU/cm^2$ for TFF, FBF or F divided by $Log_{10}CFU/cm^2$ POS. For example, for TFF this extraction efficacy ratio is > 0.94 for both *P. aeruginosa* and *E. faecalis* and for F it is 0.69 and 0.72 for *P. aeruginosa* and *E. faecalis*, respectively.

[1] TFF; Turbulent Fluid Flow extraction

[2] FBF; Flush-brush-flush extraction

[3] POS; Positive control using destructive extraction

[4] F; Flush extraction

[5] STD; standard deviation

[6] TFF extraction significantly better compared to F extraction **($p < 0.05$)**.

[7] TFF extraction trending to significantly better compared to F extraction **($p = 0.06$)**

Table 2 Extraction Efficacy of Turbulent Fluid Flow sample collection for inoculated colonoscope channels

Parameter	Suction Biopsy channel	Air/Water channel	Auxiliary channel
E. faecalis			
Pos Control Average Log_{10} CFU/cm^2	7.87 (0.09)	7.81 (0.06)	7.47 (0.06)
	% Efficiency extraction based on CFU/cm^2		
Experiment 1	99.13	97.69	99.92
Experiment 2	96.50	99.87	99.97
Experiment 3	99.69	99.73	99.99
Average (STD)*:	98.44 (1.39) **[***p***=0.002]	99.09 (1.00)	99.96 (0.03) **[***p***=0.021]
P. aeruginosa			
Pos Control Average Log_{10} CFU/cm^2	6.48 (0.43)	6.61 (0.11)	5.79 (0.45)
	% Efficiency extraction based on CFU/cm^2		
Experiment 1	99.51	97.78	99.97
Experiment 2	96.70	99.97	99.99
Experiment 3	99.76	99.88	99.99
Average (STD):	98.66 (1.39)	99.21 (1.01)	99.98 (0.01)
C. albicans			
Pos Control Average Log_{10} CFU/cm^2	6.32 (0.26)	6.32 (0.37)	5.85 (0.04)
	% Efficiency extraction based on CFU/cm^2		
Experiment 1	99.86	99.04	99.94
Experiment 2	98.63	99.96	99.97
Experiment 3	99.68	99.80	99.99
Average:	99.39 (0.54) **[***p***=0.020]	99.60(0.40)	99.97 (0.02) **[***p***=0.005]
Protein			
	% extraction efficacy based on μg/cm^2		
Pos Control Average μg/cm^2	1207.49 (193.26)	895.85 (49.28)	178.47 (23.01)
Experiment 1	99.94	99.22	99.77
Experiment 2	99.06	99.99	100.00
Experiment 3	99.95	99.98	99.71
Average STD:	99.65 (0.42)	99.73 (0.36)	99.83 (0.13)

	[p=0.019**]		
Carbohydrate			
Pos Control Average µg/cm^2	251.05 (18.34)	210.15 (41.78)	59.34(8.52)
	% extraction efficacy based on µg/cm^2		
Experiment 1	99.50	98.41	100.000
Experiment 2	99.48	99.82	100.000
Experiment 3	100.00	100.00	100.000
Average (STD):	99.66 (0.24) **[***p***=0.030**]	99.41 (0.71)	100.00 (0.00) **[***p***=0.001**]

*STD: standard deviation,

** Extraction efficacy of TFF significantly better than either FBF for Suction-Biopsy channel or F for Auxiliary channel [$p < 0.05$].

485 **Table 3: Extraction Efficacy of current Flush-brush-flush and Flush-only sample**
486 **collection for inoculated colonoscope channels**
487

Parameter	Suction Biopsy channel (FBF)	Air/Water channel (F only)	Auxiliary channel (F only)
E. faecalis			
Pos Control Average Log_{10} CFU/cm^2	7.90 (0.12)	7.78 (0.10)	7.36 (0.20)
	% Efficiency extraction based on CFU/cm^2		
Experiment 1	87.10	98.52	97.92
Experiment 2	81.66	98.30	94.27
Experiment 3	82.26	73.03	95.09
Average (STD*):	83.68 (2.43) **[***p***=0.002]	89.95 (11.96)	95.76 (1.56) **[***p***=0.021]
P. aeruginosa			
Pos Control Average Log_{10} CFU/cm^2	7.12 (0.42)	6.79 (0.60)	6.35 (0.73)
	% Efficiency extraction based on CFU/cm^2		
Experiment 1	97.91	93.99	94.03
Experiment 2	75.31	97.68	67.69
Experiment 3	82.92	75.41	94.46
Average (STD):	85.38 (9.39)	89.03 (9.75)	85.39 (12.52)
C. albicans			
Pos Control Average Log_{10} CFU/cm^2	6.50 (0.11)	6.31 (0.26)	6.04 (0.30)
	% Efficiency extraction based on CFU/cm^2		
Experiment 1	92.31	99.31	98.41
Experiment 2	80.44	99.46	98.12
Experiment 3	87.30	67.43	96.08
Average:	86.68 (4.87) **[***p***=0.020]	88.73 (15.07)	97.54 (1.04) **[***p***=0.005]
Protein			
Average Total µg/cm^2	823.51 (94.53)	795.99 (27.37)	379.97 (33.79)
	% extraction efficacy based on µg/cm^2		
Experiment 1	94.59	99.00	99.57
Experiment 2	88.74	99.64	98.30
Experiment 3	94.13	92.55	98.54
Average:	92.49 (2.66)	97.06 (3.20)	98.80 (0.55)

	[p=0.019**]		
Carbohydrate			
Average Total µg/cm^2	189.81 (12.40)	154.28 (24.49)	73.0 (21.41)
	% extraction efficacy based on µg/cm^2		
Experiment 1	91.51	99.07	98.93
Experiment 2	85.37	99.54	98.56
Experiment 3	90.36	74.42	98.47
Average:	89.08 (2.67) **[***p***=0.030**]	91.01 (11.73)	98.66 (0.20) **[***p***=0.001**]

*STD: standard deviation

** Extraction efficacy of TFF significantly better than either FBF for Suction-Biopsy channel or F for Auxiliary channel.

Table 4 Negative control culture results for colonoscope after HLD and storage

		TFF					**FBF or F**				
		CFU/cm^2					CFU/cm^2				
		Exp 1	Exp 2	Exp 3	AVE	STD	Exp 1	Exp 2	Exp 3	AVE	STD
SB	***E. faecalis***	0.019	0.000	0.005	0.008	0.008	0.000	0.000	0.000	0.000	0.000
FBF	***P. aeruginosa***	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	C. albicans	0.000	0.005	0.000	0.002	0.002	0.000	0.000	0.000	0.000	0.000
	Env. isolates	0.014	0.005	0.000	0.006	0.006	0.005	0.000	0.000	0.002	0.002
AW	***E. faecalis***	0.014	0.000	0.003	0.006	0.006	0.000	0.000	0.000	0.000	0.000
F	***P. aeruginosa***	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	C. albicans	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Env. isolates	0.003	0.000	0.000	0.001	0.002	0.000	0.000	0.000	0.000	0.000
AUX	***E. faecalis***	0.006	0.000	0.146	0.051	0.068	0.000	0.000	0.032	0.011	0.015
F	***P. aeruginosa***	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	C. albicans	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Env. isolates	0.013	0.000	0.357	0.123	0.165	0.000	0.000	0.369	0.123	0.174

HLD: High level disinfection

TFF: Turbulent fluid flow, FBF: Flush-brush-flush, F: Flush only

Exp: Experiment

AVE: Average

STD: Standard deviation

Env. isolates: Environmental isolates

References:

1. Alfa MJ, Singh H, Nugent Z, Duerksen D, Schultz G, Reidy C, DeGagne P, Olson N. Sterile reverse osmosis water combined with friction are optimal for channel and lever cavity sample collection of flexible duodenoscopes. Front Med Gastroenterol 2017a doi: 10.3389/fmed.2017.00191
2. Alfa MJ, Singh H, Nugent Z, Duerksen D, Schultz G, Reidy C, DeGagne P, Olson N. Simulated-use polytetrafluorethylene biofilm model: repeated rounds of complete reprocessing lead to accumulation of organic debris and viable bacteria. Infect Control Hosp Epidemiol 2017b http://dx.doi.org/10.1016/j.gie.2017.05.014
3. Alfa MJ, Olson N. Physical and composition characteristics of clinical secretions compared with test soils used for validation of flexible endoscope cleaning. J Hosp Infect 2016;93:83-88 DOI:10.1016/j.jhin.2016.01.023
4. Alfa MJ, Degagne P, Olson N. Worst-case soiling levels for patient-used flexible endoscopes before and after cleaning. Am. J. Infect Control 1999:27:392-401
5. ANSI/AAMI ST91:2015 Flexible and semi-rigid endoscope processing in health care facilities. American National Standards Institute Inc, Association for the Advancement of Medical Instrumentation (publisher). ISBN 1-57020-585-X
6. AS/NZS 4187:2014 Reprocessing of reusable medical devices in health service organization. Australian/New Zealand Standard
7. Aumeran C, Thilbert E, Chapelle FA, Hennequin C, Lesens O, Traore O. Assessment of experimental bacterial biofilms in clinical practice of the efficacy of sampling solutions for microbiological testing of endoscopes. J Clin Microbiol 2012;50:938–42. doi:10.1128/JCM.06221-11
8. Beilenhoff U, Neumann CS, Rey JF, Biering H, Blum R, Schmidt V, Committee EG. ESGE-ESGENA guideline for quality assurance in reprocessing: microbiological surveillance testing in endoscopy. Endoscopy 2007;**39:**175–181. http://dx.doi.org/10.1055/s-2006-945181.
9. Brock AS, Steed LL, Freeman J, Garry B, Malpas P, Cotton P. Endoscope storage time: assessment of microbial colonization up to 21 days after reprocessing. Gastrointest Endosc 2015;81:1150-4.
10. Carbonne A, Thiolet JM, Fournier S, Fortineau N, Kassis-Chikhani N, Boytechev I, Aggoune M, Seguier JC, Senechal H, Tavolacci MP, Coignard B, Astagneau P, Jarlier V. Control of a multihospital outbreak of KPC-producing *Klebsiella pneumoniae* type 2 in France, September to October 2009. Euro Surveill 2010;15(48):pii=19734. https://doi.org/10.2807/ese.15.48.19734-en.
11. Cattoir L, Vanzieleghem T, Florin L, Helleputte T, De Vos M, Verhasselt B, Boelens J, Leroux-Roels I. Surveillance of endoscopes: comparison of different sampling techniques. Infect Control Hosp Epidemiol 2017;38:1062–9. doi:10.1017/ ice.2017.115
12. Devereaux BM, Athan E, Brown RB, Greig, SM, Jones DM, Bailey FK, Wallis DJ, Singh R. Australian infection control in endoscopy consensus statements on carbapenemase-producing Enterobacteriaceae J Gastroenterol Hepatol. 2019 Apr;34(4):650-658. doi: 10.1111/jgh.14511.
13. ECRI Institute. 2015. ECRI institute recommends culturing duodenoscopes as a key step to reduce CRE infections. http://www.ecri.org/cre.

14. Epstein l, Hunter JC, Arwady MA, Tsai V, Stein L, Gribogiannis M, Frias M, Guh AY, Laufer AS, Black S, Pacilli M, Moulton-Meissner H, Rasheed JK, Avillan JJ, Kitchel B, Limbago BM, MacCannell D, Lonsway D, Noble-Wang J, Conway J, Conover C, Vernon M, Kallen AJ. New Delhi metallo-β-lactamase-producing carbapenem-resistant *escherichia coli* associated with exposure to duodenoscopes. J Am Med Assoc 2014;312:1447–55.
15. FDA Guidance; Reprocessing Medical Devices in Healthcare settings: Validation methods and labeling. Guidance for Industry and Food and Drug Administration staff. Issued March 17 2015. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.
16. FDA-CDC-ASM. Duodenoscope surveillance sampling & culturing; Reducing the risks of infection. Feb 2018.
17. Gazdik MA, Coombs J, Burke JP, Lopansri BK. Comparison of two culture methods for use in assessing microbial contamination of duodenoscopes. J Clin Microbiol 2016;54:312-316.
18. Higa JT, Gluck M, Ross AS. Duodenoscope-associated bacterial infections: a review and update. Curr Treat Options Gastroenterol 2016;14:185–93. doi:10.1007/s11938-016-0088-9
19. Ji X-Y, Ning P-Y, Zhang W, Yang D-J, Liu H, Liu J, Song J, Fei C-N. Microbiologic assessment of flexible gastrointestinal endoscope reprocessing using a pump-assisted sampling technique: an investigation involving all endoscopy units in Tianjin, China. Am J Infect Control 2018;46:e43-e48. https://doi.org/10.1016/j.ajic.2018.04.216
20. Johani K, Hu H, Santos L, Schiller S, Deva AK, et al., Determination of bacterial species present in biofilm contaminating the channels of clinical endoscopes. Infect Dis & Health 2018. https://doi.org/10.1016/j.idh.2018.06.003
21. Kola A, Piening B, Pape UF, *et al*. An outbreak of carbapenem-resistant OXa-48 - producing *Klebsiella pneumonia* associated to duodenoscopy. Antimicrob Resist Infect Control 2015;4:8.
22. Labib ME, Dukhin S, Murawski J, Tabani Y, Lai R. Surfactant influence on rivulet droplet flow in minitubes and capillaries and its downstream evolution. Adv Coll Int Sci 2011;166:60–86. doi:10.1016/j.cis.2011.05.004
23. Lui E, Lau YL, Chau YK, Pacepavicius G. Simple technique for estimation of biofilm accumulation. Bull Environ Contam Toxicol 1994;53:913e918.
24. Ma GK, Pegues DA, Kochman ML, Alby K, Fishman NO, Saunders M, Grous C, Dempsey DT, Ginsberg GG. Implementation of a systematic culturing program to monitor the efficacy of endoscope reprocessing: outcomes and costs. Gastrointest Endosc 2018;87:104-9.
25. Murray P. Preventable Tragedies: Superbugs and How Ineffective Monitoring of Medical Device Safety Fails Patients. United States: United States Senate Health, Education, Labor and Pensions Committee (2016).
26. Olafsdottir LB, Whelan J, Snyder GM. A systematic review of adenosine triphosphate as a surrogate for bacterial contamination of duodenoscopes used for endoscopic retrograde cholangiopancreatography. A J Infect Control 2018;46:697-705.
27. Petersen BT. Monitoring of endoscope reprocessing: Accumulating data but best practices remain undefined. Infect Control Hosp Epidemiol 2014;35:995-997.

28. Pineau L, De Philippe E. Evaluation of endoscope cleanliness after reprocessing: a clinical-use study. Zent Steril 2013;1:22-27. .
29. Rauwers AW, Voor in ‘t holt AF, Buijs JG, De Groot W, Hansen BE, Bruno MJ, Vos MC. High prevalence rate of digestive tract bacteria in duodenoscopes: a nationwide study. Gut 2017 doi; 10.1136/gutjnl-2017-315082.
30. Shin SP, Kim WH. Recent update on microbiological monitoring of gastrointestinal endoscopes after high-level disinfection. Clin Endosc 2015;48:369–373.
31. Systchenko R, Archetti B, Canard J, Palazzo L, Ponchon T, Rey J, Sautereau D, Endoscopy CotFSoD. Guidelines of the French Society of Digestive Endoscopy: recommendations for setting up cleaning and disinfection procedures in gastrointestinal endoscopy. Endoscopy 2000;**32:** 807– 818. http://dx.doi.org/10.1055/s-2000-7710.
32. United States Food and Drug Administration. 2015. FDA safety communication: supplemental measures to enhance duodenoscope reprocessing. http://www.fda.gov/MedicalDevices/Safety/AlertsandNotices /ucm454766.htm.
33. Verfaillie CJ, Bruno MJ, Voor in ’t Holt AF, Buijs JG, Poley J-W, Loeve AJ, Severin JA, Abel LF, Smit BJ, De Goeij I, Vos MC. Withdrawal of a novel-design duodenoscope ends outbreak of a ViM-2-producing *Pseudomonas aeruginosa*. Endoscopy 2015;47:493–502.
34. Visrodia, K, Hanada Y, Pennington KM, Tosh PK, Topazian MD, Petersen BT. Duodenoscope reprocessing surveillance with adenosine triphosphate testing and terminal cultures: a clinical pilot study. Gastrointest Endosc 2017;86:180-6
35. Waller SA, Packman AI, Hausner M. Comparison of biofilm cell quantification methods for drinking water distribution systems. J Micro Methods 2018. http://dx.doi.org/10.1016/j.mimet.2017.10.013
36. Washburn R, Pietsch JJ. Assessment of test methods for evaluating effectiveness of cleaning of flexible endoscopes. Am J Infect Control 2017. https://doi.org/10.1016/j.ajic.2017.11.014