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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 10⁸ 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 (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.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Keywords

contamination; shear force; biofilm; colonoscope

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.

Colonoscopy 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 $< 6.4 \mu\text{g}/\text{cm}^2$ and $< 1.2 \mu\text{g}/\text{cm}^2$, 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 inoculated channel with 60 cc air three times. The inoculated channels were then allowed to dry at room temperature for two hours.

Turbulent Fluid Flow (TFF) sample extraction from channels:

PTFE channels containing traditional biofilm (PTFE-TBF): A 30.5 cm length of PTFE-TBF was cut using a sterile scalpel. A channel extraction apparatus (CEA) was created by connecting the PTFE-TBF segment between two flanking segments of sterile PTFE tubing that were 76.25 cm in length and had same ID (internal diameter) as the test section to make a total length of 183 cm using connectors (Figure 1). All connectors and the TFF water pump head, and bottle cap manifold were steam sterilized prior to use. A sterile sample collection bottle was attached to the sterile bottle cap manifold for channel sample collection. The two HEPA filters and one end of the CEA containing the test section were connected to the manifold. The other end of the CEA was connected to the TFF mixing chamber. 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 accordingly for different PTFE tubing ID such as 3.2-mm and 1.37-mm. After the pump was turned on, the air valve was opened to generate TFF then 100 mL of sRO water was used for each channel extraction. Once the sRO water was finished, the pump was stopped and the air valve was closed. A 3-mL aliquot of the sample in the collection bottle was stored at -20 °C for chemistry testing and then the remaining sample was used for viable count. A portion of the extracted sample was serially diluted and 0.1 mL of each dilution was spread over the surface of a CHROMagar plate and incubated aerobically at 35 °C for 24 hours. The remainder of the sample was concentrated 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 °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/cm² 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/cm²).

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 Log₁₀ CFU/cm² 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/cm², as well as µg/cm² 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: $C1/(C1+C2+C3) \times 100$ where C1 is the CFU/cm² for the first round of extraction, C2 is the CFU/cm² for the second round of extraction and C3 is the CFU/cm² 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 $\mu\text{g}/\text{cm}^2$ of protein and carbohydrate test markers from each round of extraction.

Overview of experimental testing:

PTFE channels: The extraction 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 $\mu\text{g}/\text{cm}^2$ for organic residuals) data for biofilm testing and to analyze the % extraction efficacy based on CFU/ cm^2 (or $\mu\text{g}/\text{cm}^2$ 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 coated channels was 1.0 for TFF but only 0.72 for F. Similarly, for *P. aeruginosa* the extraction ratio was similar for TFF and FBF in 3.2-mm biofilm coated channels (0.97 and 0.98, respectively) but for 1.37-mm biofilm coated channels the ratio was 0.94 and 0.69 for TFF and F respectively. The poor extraction ratio ($p < 0.001$) for F compared to the POS was also apparent for protein and carbohydrate in the 1.37-mm channels (Table 1).

For sample extraction from endoscope channels destructive testing is not possible, so TFF extraction was compared to FBF and F extraction methods as outlined in the FDA-CDC-ASM guideline for duodenoscope channel sample collection (2018). The test markers included; CFU, protein and carbohydrate. The results of this comparison are shown in Tables 2 and 3. The TFF extraction efficacy (i.e. first round of extraction) for microorganisms was >

98% for all colonoscope channels tested, whereas the FBF and F sample collection method could not achieve this level of extraction efficacy for any of the channels tested (Table 3 shows that the microbe extraction efficacy for F and FBF ranged from 83.6% to 95.8%). Overall, the TFF extraction efficacy from inoculated colonoscope channels was significantly better than FBF or F sample collection for microbial and organic markers from the SB and AUX channels with 8/15 test parameters being significantly better for TFF extraction and 0/16 test parameters being significantly better for FBF or F sample extraction (Tables 2 and 3). For the AW channel the extraction efficacy of TFF versus F was not significantly different for any of the microbial or organic markers. This is likely due to the higher variability of the FBF and F sample collection methods (i.e. higher standard deviation).

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 µg/cm² and carbohydrate < 0.45 µg/cm². 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 µg/cm². The negative controls for the colonoscope for TFF testing showed on average < 0.051 CFU/cm² of *E. faecalis*, *P. aeruginosa* or *C. albicans* (Table 4) and < 0.3 µg/cm² 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 µg/cm². 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 6.1×10^6 CFU/mL were extracted from biofilm with sonication whereas only 1×10^2 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 Lethen broth. The destructive testing positive control showed levels of *P. aeruginosa* in their biofilm (i.e. 10^7 to 10^8 CFU/cm²) similar to the CFU/cm² in the biofilm used for our evaluation. Aumeran et al. (2012) found that flushing with water and Lethen 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.

The TFF method of creating friction to extract channel samples provides an alternative to the use of bristle brushes or a pull-through device used by Cattoir et al (2017). Unlike the other methods of creating friction, TFF can be used in a closed sample collection process that does not create aerosols and reduces the risk of environmental contamination of the sample. Our data and that of Cattoir et al. (2017) further support the study by Alfa et al. (2017a) where friction was shown to be a critical factor in sample extraction from PTFE channels coated with build-up-biofilm which is more difficult to remove than traditional biofilm.

Our current study demonstrated that extraction of organic residuals such as protein and carbohydrate from biofilm coated PTFE channels was also challenging. Although many studies have been done to assess the ability of enzymatic and non-enzymatic detergents to remove organic material in biofilms (Ren et al. 2013, Stiefel et al. 2016, da Costa et al. 2016, Neves et al. 2016, Siala et al. 2017, Gonzalez et al. 2019), this aspect is not well studied in terms of extraction of endoscope channel samples to determine the efficacy of manual cleaning. The extraction efficacy of TFF was not significantly different from that of FBF for 3.2-mm PTFE channels for protein or carbohydrate but for 1.37-mm PTFE channels there was significantly more protein extracted with TFF compared to F (TFF vs F was also trending to significance with carbohydrate extraction). The authors are not aware of other published studies that evaluated extraction of organics from biofilm within PTFE channels in terms of monitoring extraction efficacy. Because TFF sample extraction is achieved using sterile RO water (i.e. no surfactants or other additives) there would be no interference with ATP assays or with quantitative assays for protein or carbohydrate.

Since destructive testing is not feasible for reusable medical devices, the extraction of residuals from narrow channels of such devices has been focused on the use of F and FBF methods for cleaning validation (Alfa et al. 2017a, Visrodia et al. 2017, Ma et al. 2018, Pineau and De Philippe 2013) and culture (e.g. Cattoir et al. 2017, FDA-CDC-ASM guideline 2018). Indeed, Cattoir et al (2017)'s review of various National guidelines for endoscope sample collection indicated that fluid volumes ranging from 1 mL to 200 mL were recommended for F or FBF sample collection. The only published alternative approach is pump-assisted flushing using 50 mL of neutralizer fluid for extraction from endoscopes (Ji et al. 2018). This method was significantly better than manual flushing for patient-used flexible endoscopes (in terms of CFU levels detected) but no simulated-use data comparing

the extraction efficacy of the pump-assisted method to the manual method was provided. Gazdik et al. (2016) also reported that in addition to flushing the instrument channel with fluid, the use of a flocked swab instead of the larger cleaning brush recommended by the CDC interim protocol, improved the recovery of *Escherichia coli* (46%), *P. aeruginosa* (80%), and *E. faecalis* (67%) from the lever recess of duodenoscopes. The need to standardize and validate the extraction methods used for sample collection from flexible endoscopes has been recognized (Rauwers et al. 2017, Cattoir et al. 2017, Gazdik et al. 2016) but many of the published studies do not provide extraction efficacy data for the sample collection method they used (Olafsdottir et al. 2018, Rauwers et al. 2017, Shin and Kim 2015, Ji et al. 2018, Ma et al. 2018). The recently released FDA-CDC-ASM guideline (2018) sample collection protocol for duodenoscopes is one of the few studies where the three main endoscope manufacturers validated the extraction efficacy of the culture protocol. The manufacturer testing using the FBF method for duodenoscopes in the FDA-CDC-ASM 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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Highlights

- Extraction of bacteria from biofilm was better by TFF compared to Flush extraction
- TFF extraction of organic markers from biofilm was superior to Flush extraction
- TFF extraction efficacy from inoculated colonoscope channels was > 98%
- TFF extraction was significantly better than F or FBF for SB and AUX channels

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
<i>E. faecalis</i>	Log ₁₀ CFU/cm ²					
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 (STD⁵):	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)
<i>P. aeruginosa</i>	Log ₁₀ CFU/cm ²					
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 (STD⁵):	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)
<i>Protein</i>	µg/cm ²					
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 (STD⁵):	7.19 (0.90)	7.98 (2.08)	14.36 (165)	11.43 ⁶ (0.65)	0.04 (0.06)	27.25 (107)
<i>Carbohydrate</i>	µg/cm ²					
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 STD⁵:	11.43 (3.88)	11.67 (5.81)	8.47 (0.39)	17.46 ⁷ (10.62)	1.62 (0.68)	14.83 (158)

The extraction efficacy ratio is calculated as Log₁₀CFU/cm² for TFF, FBF or F divided by Log₁₀CFU/cm² 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.

¹TFF; Turbulent Fluid Flow extraction

²FBF; Flush-brush-flush extraction

³POS; Positive control using destructive extraction

⁴F; Flush extraction

⁵STD; standard deviation

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

⁷TFF 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
<i>E. faecalis</i>			
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]
<i>P. aeruginosa</i>			
Pos Control	6.48 (0.43)	6.61 (0.11)	5.79 (0.45)
Average			
Log ₁₀ CFU/cm ²			
	% 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)
<i>C. albicans</i>			
Pos Control	6.32 (0.26)	6.32 (0.37)	5.85 (0.04)
Average			
Log ₁₀ CFU/cm ²			
	% 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]
<i>Protein</i>			
	% 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

Parameter	Suction Biopsy channel	Air/Water channel	Auxiliary channel
Experiment 3	99.95	99.98	99.71
Average STD:	99.65 (0.42) ^{**} [p=0.019]	99.73 (0.36)	99.83 (0.13)
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**].

Table 3:

Extraction Efficacy of current Flush-brush-flush and Flush-only sample collection for inoculated colonoscope channels

Parameter	Suction Biopsy channel (FBF)	Air/Water channel (F only)	Auxiliary channel (F only)
<i>E. faecalis</i>			
Pos Control			
Average	7.90 (0.12)	7.78 (0.10)	7.36 (0.20)
Log ₁₀ CFU/cm ²			
	% 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]
<i>P. aeruginosa</i>			
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)
<i>C. albicans</i>			
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]
<i>Protein</i>			
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

Parameter	Suction Biopsy channel (FBF)	Air/Water channel (F only)	Auxiliary channel (F only)
Average:	92.49 (2.66) ** [p=0.019]	97.06 (3.20)	98.80 (0.55)
<i>Carbohydrate</i>			
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 colonoscopy 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	<i>E. faecalis</i>	0.019	0.000	0.005	0.008	0.008	0.000	0.000	0.000	0.000	0.000
FBF	<i>P. aeruginosa</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	<i>C. albicans</i>	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	<i>E. faecalis</i>	0.014	0.000	0.003	0.006	0.006	0.000	0.000	0.000	0.000	0.000
F	<i>P. aeruginosa</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	<i>C. albicans</i>	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	<i>E. faecalis</i>	0.006	0.000	0.146	0.051	0.068	0.000	0.000	0.032	0.011	0.015
F	<i>P. aeruginosa</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	<i>C. albicans</i>	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