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Major article

Validation of adenosine triphosphate to audit manual cleaning of flexible endoscope channels

Michelle J. Alfa PhD [a,b,c,*], Iram Fatima MSc [c], Nancy Olson BSc [c]

[a] Clinical Microbiology Diagnostic Services of Manitoba, Winnipeg, MB, Canada
[b] Microbiology Laboratory, Saint Boniface Research Centre, Winnipeg, MB, Canada
[c] Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada

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Background: Compliance with cleaning of flexible endoscope channels cannot be verified using visual inspection. Adenosine triphosphate (ATP) has been suggested as a possible rapid cleaning monitor for flexible endoscope channels. There have not been published validation studies to specify the level of ATP that indicates inadequate cleaning has been achieved.
Objective: The objective of this study was to validate the Clean-Trace (3M Inc, St. Paul, MN) ATP water test method for monitoring manual cleaning of flexible endoscopes.
Methods: This was a simulated use study using a duodenoscope as the test device. Artificial test soil containing 10^6 colony-forming units of *Pseudomonas aeruginosa* and *Enterococcus faecalis* was used to perfuse all channels. The flush sample method for the suction-biopsy (L1) or air-water channel (L2) using 40 and 20 mLs sterile reverse osmosis water, respectively, was validated. Residuals of ATP, protein, hemoglobin, and bioburden were quantitated from channel samples taken from uncleaned, partially cleaned, and fully cleaned duodenoscopes. The benchmarks for clean were as follows: $<6.4\ \mu g/cm^2$ protein, $<2.2\ \mu g/cm^2$ hemoglobin, and <4-$\log_{10}$ colony-forming units/cm^2 bioburden.
Results: The average ATP in clean channel samples was 27.7 RLUs and 154 RLUs for L1 and L2, respectively (<200 RLUs for all channels). The average protein, hemoglobin, and bioburden benchmarks were achieved if <200 RLUs were detected. If the channel sample was >200 RLUs, the residual organic and bioburden levels would exceed the acceptable benchmarks.
Conclusion: Our data validated that flexible endoscopes that have complete manual cleaning will have <200 RLUs by the Clean-Trace ATP test.

Reprocessing of flexible endoscopes includes manual cleaning in most health care facilities offering endoscopic procedures.[1] The manual cleaning phase has been shown to be the process most prone to human error. Aumeran et al[2] recently reported an outbreak involving transmission of a multiresistant *Klebsiella pneumonia* that was linked to inadequate cleaning and drying of endoscopic retrograde choliangiopancreatography (ERCP) endoscopes. Despite identifying the manual cleaning as a key concern, most published audits have sampled endoscopes after they have been high-level disinfected.[3-7] An audit tool that would allow facilities to proactively assess compliance with the manual cleaning phase of flexible endoscope reprocessing would be valuable for training as well as ongoing monitoring.[8-12] The only commercially available validated rapid test that can be used by health care facilities to evaluate whether adequate cleaning of flexible endoscope channels has been performed is the "Channel Check" (HealthMark Industries Inc, Detroit, MI) test for residual organic material. Culturing is another approach that has been recommended to evaluate the level of bacteria in the endoscope channels immediately after complete reprocessing or after storage.[3,4,6,7] This is an appropriate parameter to measure but is not feasible for many health care facilities with no access to a microbiology laboratory. Furthermore, the results of the

* Address correspondence to Michelle J. Alfa, PhD, Diagnostic Services of Manitoba, St. Boniface Hospital site, 409 Tache Avenue, Winnipeg, MB, Canada R2H 2A6.
E-mail address: malfa@dsmanitoba.ca (M.J. Alfa).
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Conflicts of interest: Dr. Michelle Alfa is the inventor of Artificial Test Soil, and the patent has been licensed through the University of Manitoba by Healthmark Industries. Dr. Alfa has been an invited guest speaker at many national and international conferences that were sponsored by various companies including Olympus, 3M, STERIS, J&J, Healthmark, and Virox. In addition she has provided consulting services for Olympus, 3M, STERIS, and J&J. The remaining authors disclose no conflicts.

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culture tests are not available until after the scope has been used on other patients. Adenosine triphosphate (ATP) is present in all viable prokaryotic microorganisms and eukaryotic human cells,[13] and there are several published reports indicating that ATP monitoring provides a valuable method for auditing endoscope cleaning.[9-12] However, there have been no published studies to date to validate the sample collection or the benchmark for ATP that correlates with effective cleaning for endoscope channels.

One objective of this study was to assess various approaches for sample collection from endoscope channels and determine the optimal method. A second objective was to use the optimal channel sample collection method and use simulated-use testing to validate the relative light units (RLU) benchmark that can be achieved after complete manual cleaning.

MATERIALS AND METHODS

Organic challenge: Artificial test soil

Freshly prepared Artificial Test Soil (Artificial Test Soil: US patent 6,447,990) was used as the organic challenge for soiling flexible endoscope channels.[14,15] The test soil was freshly prepared and contained ATP (Note: the lyophilized commercial product does not contain much ATP).

Bacteria

The organisms used for simulated-use testing included *Enterococcus faecalis* (ATCC 29212) and *Pseudomonas aeruginosa* (ATCC 27853).

Flexible endoscope used for simulated-use testing

An Olympus duodenoscope model JF-Type 140F (Olympus America Inc, Center Valley, PA) was used for all simulated-use testing. The entire length from the umbilical to distal end of the suction-biopsy channel (L1), and the air-water channel (L2), was perfused with Artificial Test Soil containing 10^8 colony-forming units (cfu)/mL of each test microorganism. After a 1-hour dry time, various levels of cleaning were performed, and then the entire channel length was sampled. Special tubing segments that allowed connection of a syringe to the outlets on the umbilical portion of the endoscope as well as plastic plugs for the control head valve openings were used for the channel inoculation and harvesting procedures. For all L1 and L2 channels harvesting, a total of 40 mLs and 20 mLs of sterile reverse osmosis (sRO) water, respectively, was flushed through the channel to extract any residual organic material and bioburden. Various methods of harvesting L1 were evaluated including:

1. Flush-brush-flush: 20 mLs of sRO water was flushed through the channel, followed by brushing up and down 3 times with a sterile channel brush (STERIS Inc, Mentor, OH), cutting the brush end off into the sample collection container, followed by flushing the remaining 20 mL sRO water through the channel. The 40 mLs of sRO water and the brush were pooled as the sample used for analysis.
2. Flush-sponge-flush: this method is identical to the Flush-brush-flush method except that an Endozime Instrusponge (Ruhof Corp., Mineola, NY) was used in place of the channel brush.
3. Flush: this method of channel harvesting consisted of a single flush 40 mLs sRO water slowly through the L1 channel.

The L2 channel was sampled using a flush of 20 mLs sRO water.

Assay methods for ATP, protein, hemoglobin, and bioburden quantitation

The Clean-Trace ATP water test kit (3M Inc, St. Paul, MN) was used for channel (liquid) samples. The RLU measurement of ATP in each channel sample was determined using the handheld Biotrace luminometer (3M Inc) as per the manufacturer's instructions. All experiments were performed in triplicate, and results were presented as the average RLUs/sample.

Protein was measured using the QuantiPro BCA assay kit, which includes a bovine serum albumin protein standard and is a quantitative assay based on bicinchoninic acid (Sigma, St. Louis, MO). The 3,3′,5,5′ tetramethylbenzadine Liquid substrate system for enzyme-linked immunosorbent assay (Sigma) was used in conjunction with a 80 mg/dL cyanmethemoglobin standard (Stanbio Laboratory, Boerne, TX) for hemoglobin quantitation. The hemoglobin and protein assays were performed as per the manufacturers' instructions and had limits of detection of 5 μg/mL and 0.5 μg/mL, respectively.

The bioburden quantitation was performed using standard serial 1:10 dilutions with the spread plate method using 0.1 mLs of each dilution onto BBL CHROMagar Orientation media (BD Biosciences, Mississauga, ON). The limit of detection for the viable count assay was 10 cfu/mL.

Benchmarks for adequate manual cleaning

The manual cleaning benchmarks for flexible endoscope channels that were established by Alfa et al[14] were used. If manual cleaning has been adequate, then there should be <6.4 μg/cm^2 of protein, <2.2 μg/cm^2 of hemoglobin, and <4-log$_{10}$ cfu/cm^2 of bioburden.

RESULTS

The average volume (5 replicates) of fluid sampled using the ATP collection device was 0.122 mLs. The ATP level in potable tap water was 255.4 ± 97.7 RLUs and for sRO water was 20 ± 13.1 RLUs (5 replicates). Our limit of detection testing showed that to get 1 RLU using this ATP assay requires $\sim10^3$ cfu/mL of *Enterococcus faecalis* and $\sim10^2$ cfu/mL of *Pseudomonas aeruginosa*. The routine channel brush as well as the Endozime Instrusponge could be used for collection of endoscope channel samples for ATP testing because the average endosponge and brush baseline values were 18.7 RLUs and 16 RLUs, respectively.

To assess what harvesting method for L1 was optimal, repeat rounds of harvesting were performed using flush only (FO), flush-brush-flush (FBF), or flush-sponge-flush (FSF) harvesting. The data for L1 indicate that the FO harvesting method provided slightly better recovery of protein, hemoglobin, ATP, and viable organisms from the inoculated channel compared with the FBF or FSF methods. Furthermore, repeated rounds using the FO method for L1 (Fig 1) demonstrated that 85% to 100% of recoverable protein, hemoglobin, ATP, and viable organisms were obtained in the first round of harvesting. Repeating the harvesting did not improve the efficiency enough to warrant more than 1 round of channel harvesting. The results for L2 were similar (data not shown). Based on these findings, all subsequent testing used 1 round of harvesting with the FO method for sampling of L1 and L2.

To assess how well residual ATP correlated with organic and bioburden residuals after manual cleaning, the FO harvesting method was used to collect samples from L1 and L2. The soiled scopes were evaluated after no cleaning, partial cleaning (consisting of flushing 50 mLs of sterile tap water through each channel), or complete cleaning (as per the manufacturer's instructions). Each

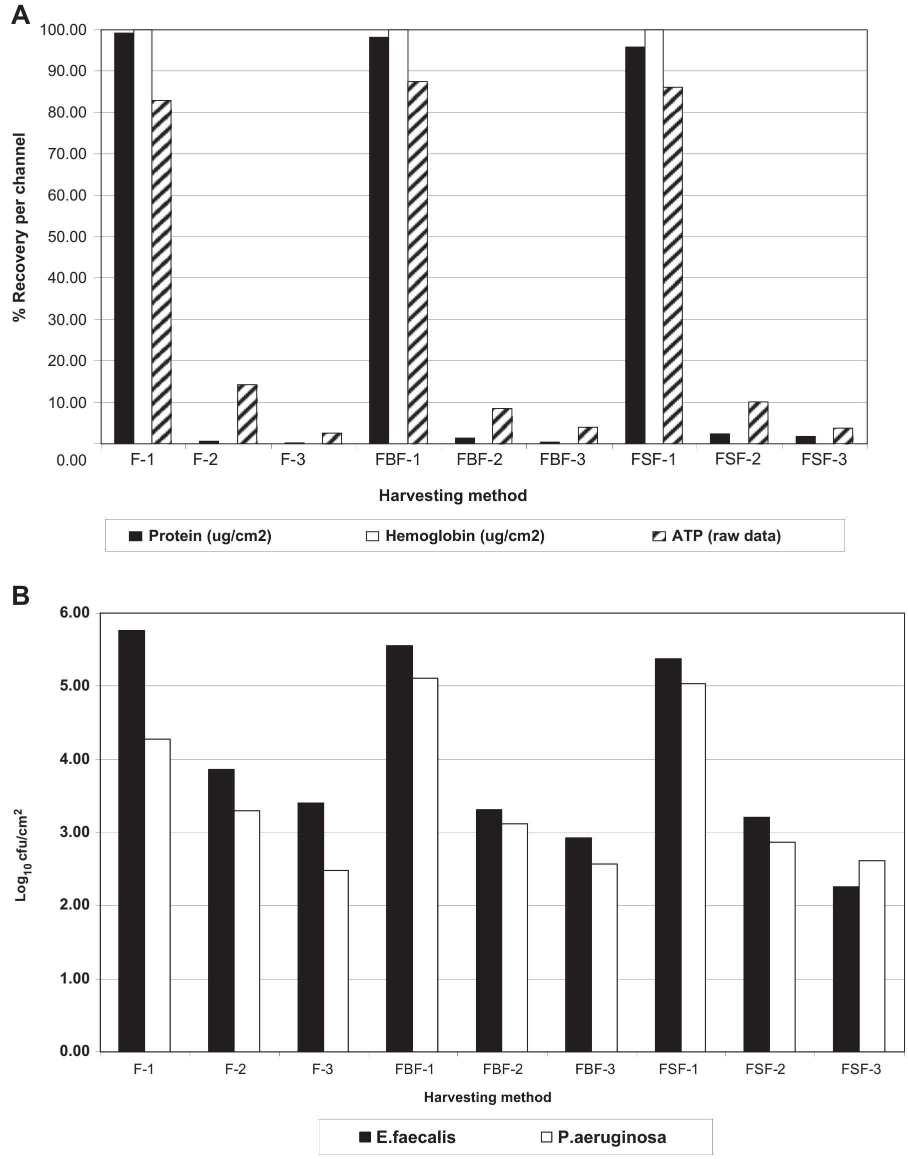

Fig 1. Preliminary testing to determine optimal method for harvesting the L1 suction biopsy channel of a duodenoscope. The methods evaluated included flush only (FO), flush-brush-flush (FBF), and flush-sponge-flush (FSF). The harvesting method was repeated 3 consecutive times to determine multiple rounds of harvesting were needed. F-1 indicated the first round of harvesting, F-2 the second, and F-3 the third consecutive round of harvesting for the same channel. The harvested samples were quantitatively assayed for protein, hemoglobin, and ATP as shown in (A), and the viable count data are shown in (B). Each bar represents the average of 3 replicate experiments.

Table 1
Simulated-use assessment of ATP, protein, hemoglobin, and bioburden to evaluate cleaning efficacy in a duodenoscope

	ATP, RLU/sample (SD)	Protein, μg/cm^2 (SD)	Hemoglobin, μg/cm^2 (SD)	*Enterococcus faecalis*, $\log_{10}$ cfu/cm^2 (SD)	*Pseudomonas aeruginosa*, $\log_{10}$ cfu/cm^2 (SD)
Not cleaned (positive control)					
L1	18,611.67 (1,939.32)	305.19 (32.28)	30.05 (4.53)	6.45 (0.09)	6.00 (0.07)
L2	19,818.00 (7,622.12)	568.20 (317.02)	27.12 (13.27)	6.50 (0.29)	6.20 (0.26)
Total clean					
L1	27.67 (15.31)	0.10 (0.07)	0.63 (0.00)	2.37 (0.19)	2.21 (0.37)
L2	154.00 (26.85)	0.22 (0.18)	0.72 (1.25)	3.18 (1.06)	3.11 (1.29)
Partial clean					
L1	9,385.33 (1,154.18)	57.17 (4.90)	4.56 (5.00)	5.76 (0.09)	5.27 (0.37)
L2	24,560.67 (18,975.83)	155.62 (173.51)	16.30 (14.71)	5.93 (0.25)	5.22 (0.68)
Negative control (unused)					
L1	18.33 (15.37)	0.04 (0.07)	0.63 (1.09)	0.00 (0.00)	0.00 (0.00)
L2	9.67 (2.08)	0.07 (0.07)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

L1, Suction-biopsy channel; *L2*, air-water channel; *SD*, standard deviation.
NOTE. Results for all assays represent the average of 3 replicate experiments. The channels were sampled using the FO method. A value of 0 denotes < limit of detection. The protein assay limit of detection is 0.5 μg/mL, the hemoglobin assay limit of detection is 5.0 μg/mL, and the bioburden limit of detection is 10 cfu/mL.

channel sample obtained was assayed for total ATP, protein, hemoglobin, and viable count. The overall results for the simulated-use testing are shown in Table 1.

The research technologist indicated that it takes less than 2 minutes to flush fluid through 1 channel to collect the sample and approximately 1 minute to perform the ATP test. For a scope with 2 channels (eg, colonoscope), the entire process for collecting sample samples from both channels and performing the ATP test would be less than 4 minutes.

DISCUSSION

Currently, health care standards recommend that medical devices be "visibly clean,"[1,8] but this is inadequate for lumens of flexible endoscopes because they cannot be visualized. Although measurement of residual organic material (eg, protein) and/or viable counts have been used, the assay results are not available until 24 to 48 hours later, and the scope may have already been used on another patient. The food industry has used ATP to monitor environmental cleaning for many years; however, the application of this method to health care has only recently been undertaken.[9-12] Our study is the first to validate the sampling method and the RLU level that correlates with adequate endoscope cleaning.

Obee et al[10] evaluated ATP testing as a method to monitoring cleaning of flexible endoscope lumens. The benchmark they used was 500 RLUs; however, they did not validate this ATP benchmark with respect to residual microbial and organic levels within fully cleaned channels.[10] Similarly, the study by Hansen et al[9] attempted to establish an endoscope application for ATP testing using the Lumitester PD 10 (Schil Diagnostics, Selangor, Malaysia). In their study, only the suction-biopsy channel was evaluated, and they did not validate the benchmark for the cleaning stage; rather, they sampled the endoscopes after being fully reprocessed. None of the published endoscope channel studies[9,10] to date have validated the channel harvesting method that was used. The brushing step for L1 during the manual cleaning process for patient-used endoscopes is still required, but our data indicate that, after manual cleaning, there is no need to use a brush or sponge for channel sampling because it does not improve sample recovery compared with the FO method. This likely reflects the efficiency of sRO water in "stripping" organic material and organisms off the inner surface of the manually cleaned endoscope channel. For all other channels in the scope, there is no choice but to use the FO method because most do not have channel brushes that are designed for these smaller channels.

Our data confirm Turner et al[13] and Aiken et al's[11] findings and demonstrated that the limit of detection (ie, lowest level of organisms that would generate 1 RLU using the Clean-Trace ATP test kit; 3M Inc) for viable bacteria was $\sim 10^2$ cfu/mL and 10^3 cfu/mL of gram-negative and gram-positive organisms, respectively.

Using simulated-use testing, we demonstrated that complete manual cleaning of soiled duodenoscope channels effectively reduced the organic and bioburden residuals to below the acceptable limits as defined by TIR30[5] (ie, <6.4 μg/cm^2 protein, <2.2 μg/cm^2 hemoglobin, and <4-log$_{10}$ cfu/cm^2) and resulted in an average of <200 RLUs when tested using the ATP assay (ranges, 16-45 RLUs for L1 and 130-183 RLUs for L2). Furthermore, the residual RLUs for samples taken from partially cleaned endoscope channels correlated well with traditional quantitative protein, hemoglobin, and bioburden assays in that the ATP assay flagged the cleaning as inadequate (ie, >200 RLUs) as did the other assays. Our data (Table 1) indicated that L2 was harder to clean because there were more residuals remaining in L2 compared with L1 despite total cleaning. This is most likely because there is no physical brushing of this channel during the manual cleaning process.

Based on our validation study, we recommend that, for the FO harvest method, an RLU benchmark of 200 would provide assurance that the published benchmarks[5,8] for cleaning of flexible endoscopes of <6.4 μg/cm^2 protein, <2.2 μg/cm^2 hemoglobin, and <4-log$_{10}$ cfu/cm^2 have been achieved for both L1 and L2. It should be noted that this benchmark of <200 RLUs is a measure of combined ATP from organic and bioburden residuals, and, although it is appropriate for the Clean-Trace ATP test kit (3M Inc), it may not be applicable to other manufacturers' ATP test kits because Aiken et al[11] have shown that different manufacturers' ATP test kits have differing levels of RLUs when tested against the same number of bacteria.

CONCLUSION

The results of our simulated-use study support the application of the ATP method as a reliable means of monitoring manual cleaning efficacy on a "real-time" basis for channels of flexible endoscopes. Further clinical studies are warranted to confirm that the 200 RLU benchmark for endoscope lumen samples can be reliably achieved in busy clinic settings.

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