Major article

The adenosine triphosphate test is a rapid and reliable audit tool to assess manual cleaning adequacy of flexible endoscope channels

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

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

Background: The study objective was to verify that the adenosine triphosphate (ATP) benchmark of <200 relative light units (RLUs) was achievable in a busy endoscopy clinic that followed the manufacturer's manual cleaning instructions.
Methods: All channels from patient-used colonoscopes (20) and duodenoscopes (20) in a tertiary care hospital endoscopy clinic were sampled after manual cleaning and tested for residual ATP. The ATP test benchmark for adequate manual cleaning was set at <200 RLUs. The benchmark for protein was <6.4 $\mu g/cm^2$, and, for bioburden, it was <4-$\log_{10}$ colony-forming units/cm^2.
Results: Our data demonstrated that 96% (115/120) of channels from 20 colonoscopes and 20 duodenoscopes evaluated met the ATP benchmark of <200 RLUs. The 5 channels that exceeded 200 RLUs were all elevator guide-wire channels. All 120 of the manually cleaned endoscopes tested had protein and bioburden levels that were compliant with accepted benchmarks for manual cleaning for suction-biopsy, air-water, and auxiliary water channels.
Conclusion: Our data confirmed that, by following the endoscope manufacturer's manual cleaning recommendations, 96% of channels in gastrointestinal endoscopes would have <200 RLUs for the ATP test kit evaluated and would meet the accepted clean benchmarks for protein and bioburden.
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Although there are automated endoscope reprocessors that have validated cleaning cycles[1,2] with various levels of US Food and Drug Administration clearance, most health care facilities still use manual cleaning for reprocessing of flexible endoscopes.[3,4] Manual cleaning has been reported to be prone to human error,[4] and a recent report by Aumeran et al[5] indicated that an outbreak of a multiresistant *Klebsiella pneumonia* was linked to transmission because of inadequate cleaning and drying of endoscopic retrograde cholangiopancreatography (ERCP) endoscopes. There is need for a rapid audit tool for ongoing quality assurance monitoring that

would allow facilities to proactively assess compliance with the manual cleaning phase of flexible endoscope reprocessing.[6-12] The only commercially available validated rapid test that endoscopy clinics could use to evaluate the adequacy of channel cleaning for flexible endoscopes is the "Channel Check" (HealthMark Industries Company Inc, Detroit, MI) test for residual organic material (blood, protein, and carbohydrate). One other approach that has recently been adapted to monitor flexible endoscopes[8-11] is the use of adenosine triphosphate (ATP) as a measure of cleaning adequacy. ATP is present in microorganisms as well as human cells.[7] As such, relative light units (RLUs) detected after cleaning could represent residual bioburden or patient secretions that contain cellular ATP. Several groups have reported[7,10,13] that ATP tests require a high bioburden level before a strong RLU signal will be detected. Alfa et al[13] have reported that to detect 1 RLU, the sample would need to contain $\sim 10^3$ colony-forming units (cfu) of a gram-positive organism or 10^2 cfu of a gram-negative organism. As reported by Turner et al,[7] the relationship between RLUs and colony-forming units is not linear. Although there are published reports indicating that ATP monitoring provides a valuable method for auditing endoscope cleaning,[8-11] these studies did not validate the channel

harvesting method or the benchmark for residual ATP RLUs that correlated with effective cleaning for endoscope channels. A recent study in our laboratory[13] has validated a channel harvesting method as well as a target level of 200 RLUs (when using the Clean-Trace ATP water test, Minneapolis, MN) that should be achievable in channels of adequately cleaned flexible endoscopes. The objective of the current study was to verify that the ATP benchmark of <200 RLUs established for manual cleaning using simulated-use testing is achievable in a busy endoscopy clinic that is following the manufacturer's manual cleaning process.

MATERIALS AND METHODS

Flexible endoscopes and sample method used for clinical testing

Consecutive, patient-used endoscopes were selected, and the only exclusion criteria was that endoscopes used after regular clinic hours would not be included in this study (because study personnel were not available after hours). Patient-used colonoscopes and duodenoscopes were sampled before or after manual cleaning as well as after complete reprocessing, which consisted of manual cleaning and high-level disinfection (HLD). The endoscopes used for this clinical study were all manufactured by Olympus America Inc (Center Valley, PA) and included video-colonoscopes model CF-Q180AL, video-colonoscopes model CF-H180AL, and video-duodenoscopes model TJF-160VF. There were several of each model of endoscope used in the clinic, and each had a unique identifying number. 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 harvesting. All connection tubing and plugs were cleaned and steam sterilized for each sample collection. For all channel harvesting, the flush-only method validated by Alfa et al[13] was used whereby 40 mLs, 20 mLs, 10 mLs, 5 mLs of sterile reverse osmosis (RO) water were flushed through the suction-biopsy (L1), air-water (L2), auxiliary water (L3), and elevator guide-wire (L4) channels, respectively, to extract any residual organic material and bioburden. For the suction-biopsy, air-water, and auxiliary water channels, the RO water used for sample collection was flushed from the umbilical end to the distal end, whereas, for the elevator guide wire, the sample was flushed from the control head to the distal end. Each endoscope that was used for a patient procedure received a bedside external wipe, and all channels were flushed with Renuzyme (Getinge, Mississauga, ON, Canada) at the use-dilution recommended by the detergent manufacturer, and the scope was then transported to the endoscopy reprocessing room. All scopes were transported and had manual cleaning performed within 1 hour. The written procedures used for manual cleaning reflected the endoscope manufacturer's instructions and included leak testing, immersion in enzymatic detergent at the appropriate temperature for the appropriate contact time, brushing of channels, flushing of detergent through the channels, and a tap water rinse followed by HLD. A preliminary observational audit confirmed that the dedicated reprocessing technician was following the manual cleaning steps outlined by the endoscope manufacturer (Olympus America Inc). This site used an EFP 250 Endo-Flush endoscope flushing pump (Olympus America Inc) to facilitate flushing of detergent through the channels after the channel brushing has been completed. The channel flush consisted of 1.25 L shared between the suction-biopsy and air-water channels and a 0.2 L flush for the auxiliary or elevator guide-wire channel (if present). The endoscope was transferred to a basin of fresh tap water, and the final tap water rinse consisted of the same volumes as indicated for the detergent flush. The endoscopes were disinfected using 2% glutaraldehyde (MetriCide from Metrex Inc, Romulus, MI) for 20 minutes at room temperature in

a Medivator (Minntech, Minneapolis, MN) automated endoscope reprocessor. Prior to storage all endoscope channels were rinsed with ethanol, and forced air was used to dry the channels. Endoscopes were hung in a locked cabinet.

Assay methods for ATP, protein, and viable organisms

The Clean-Trace ATP water test (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. The cutoff for adequate cleaning (channels) was set at <200 RLUs because this was validated for the channel harvesting method that was used.[13] There were 10 patient-used endoscopes that were tested post-patient use prior to manual cleaning and 20 patient-used endoscopes that were evaluated after manual cleaning. In addition to the samples taken before and after manual cleaning, there were samples collected from 10 unused endoscopes. These unused endoscopes had been manually cleaned, had received HLD, and had been stored over the weekend and were sampled on Monday morning just prior to patient use. The results were presented as the average RLUs/sample.

In addition to ATP testing, a portion of each endoscope channel sample was also assayed for protein and bioburden. For all samples, protein was assessed using the QuantiPro BCA assay kit (Sigma, St. Louis, MO) that includes a bovine serum albumin protein standard and is a quantitative assay based on bicinchoninic acid. The bioburden quantitation was performed using standard serial 1:10 dilutions with the spread plate method where 0.1 mL of each dilution was inoculated onto blood agar medium. 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[14,15] were used. If manual cleaning has been adequate, there should be <6.4 $\mu g/cm^2$ of protein and <4-$\log_{10}$ cfu/cm^2 of bioburden.

Statistical analysis

All data were entered into an Excel software (Microsoft Corp, Redmond, WA) spreadsheet. The study results were analyzed by a 2-tailed t test using GraphPad InStat (GraphPad Software Inc, La Jolla, CA).

RESULTS

This study was performed in a 600-bed, Canadian acute care teaching hospital in an endoscopy clinic that does approximately 40 gastrointestinal endoscopy procedures per day. Each channel in the flexible endoscope was harvested using the sterile RO water flush-only sampling protocol previously validated.[13] The ATP, protein, and bioburden residuals detected after manual cleaning by the clinic reprocessing staff are summarized in Tables 1, 2, and 3, respectively. Each Table indicates the level of residuals pre- and postcleaning as well the number of scopes evaluated that exceeded the benchmarks for clean.

The data from unused endoscopes (Tables 1-3) represent samples taken from flexible endoscopes that have been completely reprocessed using manual cleaning and HLD. This testing of unused endoscopes indicated that, although all channels from 10 colonoscopes and 10 duodenoscopes were below the benchmarks for ATP, protein, and bioburden (Tables 2 and 3), there was 1 duodenoscope elevator guide wire that exceeded the ATP benchmark of 200 RLU/sample. In addition, 25% of the L4 channel tests exceeded the

Table 1
ATP levels in channels of patient-used colonoscopes and duodenoscopes before and after manual cleaning

	Endoscope channels tested			
	L1 Suction-biopsy	L2 Air-water	L3 Auxillary water	L4 Elevator guide-wire channel
Average ATP residual level RLUs/sample (standard deviation) Colonoscopes:				
Preclean (n = 10)	1,315.8 (1,507.7)	39.3 (47.6)	17.5 (8.3)	Channel not present
Postclean (n = 20)	20.4 (29.9)	15.2 (7.3)	12.1 (5.9)	Channel not present
Unused (n = 10)*	25.5 (21.4)	13.2 (4.2)	13.1 (4.0)	Channel not present
Duodenoscopes:				
Preclean (n = 10)	10,667.2 (29,106.1)	102.1 (127.4)	Channel not present	2,430.0 (3,148.1)
Postclean (n = 20)	47.9 (45.9)	16.4 (5.4)	Channel not present	164.1 (184.9)
Unused (n = 10)*	27.7 (13.0)	13.9 (8.2)	Channel not present	136.0 (174.7)
Endoscope channels that exceeded the benchmark for adequate cleaning				
	L1	L2	L3	L4
Number of scopes with ≥200 RLUs/test ATP residual level (%) Colonoscopes:				
Preclean (n = 10)	9/10 (90)	0/10 (0)	0/10 (0)	Channel not present
Postclean (n = 20)	0/20 (0)	0/20 (0)	0/20 (0)	Channel not present
Unused (n = 10)*	0/10 (0)	0/10 (0)	0/10 (0)	Channel not present
Duodenoscopes:				
Preclean (n = 10)	10/10 (100)	2/10 (20)	Channel not present	7/10 (70)
Postclean (n = 20)	0/20 (0)	0/20 (0)	Channel not present	5/20 (25)
Unused (n = 10)*	0/10 (0)	0/10 (0)	Channel not present	1/10 (10)

*Unused: Fully cleaned and disinfected endoscope sampled on Monday morning after weekend storage.

Table 2
Protein levels in channels of patient-used colonoscopes and duodenoscopes before and after manual cleaning

	Endoscope channels tested			
	L1 Suction-biopsy	L2 Air-water	L3 Auxillary water	L4 Elevator guide-wire channel
Average protein residual level $\mu g/cm^2$ (standard deviation) Colonoscopes:				
Preclean (n = 10)	1.321 (0.900)	0.032 (0.83)	0.066 (0.108)	Channel not present
Postclean (n = 20)	0.030 (0.065)	0.020 (0.046)	0.008 (0.025)	Channel not present
Unused (n = 10)*	0.016 (0.038)	0.003 (0.008)	0	Channel not present
Duodenoscopes:				
Preclean (n = 10)	0.688 (0.877)	0.004 (0.013)	Channel not present	0.201 (0.270)
Postclean (n = 20)	0.044 (0.096)	0.018 (0.062)	Channel not present	0.039 (0.068)
Unused (n = 10)*	0.076 (0.066)	0.037 (0.058)	Channel not present	0.242 (0.153)
Endoscope channels that exceeded the benchmark for adequate cleaning				
	L1	L2	L3	L4
Number of scopes with ≥6.4 $\mu g/cm^2$ protein residual level (%) Colonoscopes:				
Preclean (n = 10)	0/10 (0)	0/10 (0)	0/10 (0)	Channel not present
Postclean (n = 20)	0/20 (0)	0/20 (0)	0/20 (0)	Channel not present
Unused (n = 10)*	0/10 (0)	0/10 (0)	0/10 (0)	Channel not present
Duodenoscopes:				
Preclean (n = 10)	0/10 (0)	0/10 (0)	Channel not present	0/10 (0)
Postclean (n = 20)	0/20 (0)	0/20 (0)	Channel not present	0/20 (0)
Unused (n = 10)*	0/10 (0)	0/10 (0)	Channel not present	0/10 (0)

*Unused: Fully cleaned and disinfected endoscope sampled on Monday morning after weekend storage.

benchmark of 200 RLU/sample for endoscopes that had been patient used and then manually cleaned (Table 1).

DISCUSSION

Our clinical study demonstrated that by using the validated RO water flush-only sample collection and the RLU benchmark of <200 RLUs, adequate manual cleaning was achieved in 115 of 120 (96%) of lumens tested for patient-used colonoscopes and duodenoscopes. The 5 lumens that had >200 RLUs were all elevator guide-wire channels. The 5 endoscopes were all TJF-160VF models but were different endoscopes that were used for patient procedures on 3 separate days. Indeed, it is apparent that the L4 elevator guide-wire channel is very difficult to properly clean because 1 of 10 (10%) of the elevator guide-wire channels from unused, fully reprocessed endoscopes had >200 RLUs when tested just prior to patient use. Although the protein and bioburden levels in these same samples

were within acceptable benchmarks, the RLU data indicated that cleaning did not reach what should be achievable based on simulated-use studies.[14] It is difficult to conclusively determine whether the inadequate cleaning of the L4 channel was a reflection of Ofstead et al's[4] contention that there is great variability in reprocessing staff being able to consistently achieve all steps in reprocessing for all endoscopes. Because an Endo Flush pump would have been used to clean the elevator guide wire, it is possible there were problems with the pump flushing the expected volume through the channel. However, because the same L4 channel from these endoscopes was adequately cleaned on other days, it is difficult to fully explain what the problem was (eg, the L4 channel from endoscope number 27 had 480 RLUs postcleaning on 1 occasion, but, on 3 other days, the same L4 channel had ≤30 RLUs postcleaning). Our data indicate that the L4 channel is the most difficult to clean (5/20 had >200 RLUs postcleaning) and does have high organic and bioburden levels after patient procedures. Indeed,

Table 3
Bioburden levels in channels of patient-used colonoscopes and duodenoscopes before and after manual cleaning

	Endoscope channels tested			
	L1 Suction-biopsy	L2 Air-water	L3 Auxillary water	L4 Elevator guide-wire channel
Average bioburden residual level $\log_{10}$ cfu/cm^2 (standard deviation) Colonoscopes:				
Preclean (n = 10)	2.838 (1.161)	0.120 (0.274)	0.283 (0.643)	Channel not present
Postclean (n = 20)	1.038 (1.184)	0.437 (0.607)	0.264 (0.447)	Channel not present
Unused (n = 10)*	0.082 (0.250)	0.001 (0.068)	0.002 (0.047)	Channel not present
Duodenoscopes:				
Preclean (n = 10)	1.152 (1.239)	0.186 (0.602)	Channel not present	1.688 (1.507)
Postclean (n = 20)	0.378 (0.602)	0.106 (0.277)	Channel not present	0.803 (1.115)
Unused (n = 10)*	0.004 (0.064)	0	Channel not present	0.001 (0.030)
Endoscope channels that exceeded the benchmark for adequate cleaning				
	L1	L2	L3	L4
Number of scopes with ≥4-$\log_{10}$ cfu/cm^2 bioburden residual level (%) Colonoscopes:				
Preclean (n = 10)	0/10 (0)	0/10 (0)	0/10 (0)	Channel not present
Postclean (n = 20)	0/20 (0)	0/20 (0)	0/20 (0)	Channel not present
Unused (n = 10)*	0/10 (0)	0/10 (0)	0/10 (0)	Channel not present
Duodenoscopes:				
Preclean (n = 10)	0/10 (0)	0/10 (0)	Channel not present	0/10 (0)
Postclean (n = 20)	0/20 (0)	0/20 (0)	Channel not present	0/20 (0)
Unused (n = 10)*	0/10 (0)	0/10 (0)	Channel not present	0/10 (0)

*Unused: Fully cleaned and disinfected endoscope sampled on Monday morning after weekend storage.

the average bioburden level in the L4 channel was higher than that in the L1 suction-biopsy channel both pre- and postcleaning. For ERCP endoscopes, our data suggest that the L4 (elevator guide-wire channel) should be routinely monitored for cleaning adequacy for all patient-used endoscopes.

Our data demonstrated that the bedside wipe and channel flushing was very effective at reducing the levels of organic and bioburden residuals from within the channels of patient-used duodenoscopes and colonoscopes. In the current study, the residual organic (protein) levels post-bedside flush but precleaning were significantly lower compared with those reported from Alfa et al[14] where no bedside flushing was performed. With a bedside flush, L1 had 1.321 ± 0.9 μg/cm^2 and 0.688 ± 0.877 μg/cm^2 for colonoscopes and duodenoscopes, respectively. Whereas, without a bedside flush,[14] L1 had 37.05 ± 16.926 μg/cm^2 and 11.32 ± 9.417 μg/cm^2 for colonoscopes ($P < .0001$, 95% confidence interval: 24.468-46.090) and duodenoscopes ($P = .0023$, 95% confidence interval: 4.349-16.915), respectively. Similarly, bioburden levels were significantly higher in the data reported by Alfa et al[14] where no bedside flushing was performed.

Our RLU data (Table 1) indicated that the duodenoscope L1 channel was far "dirtier" than the L1 channel of precleaned colonoscopies. This was primarily because of 2 duodenoscope L1 channels that had exceedingly high RLUs. On 2 occasions, endoscope number 25 had 93,270 and 16,312 RLUs, respectively, precleaning (ATP assay repeated to confirm these RLU levels) despite having protein that was below the limit of detection in the same samples. This suggests that the bedside flush was either not done or inadequate and that the patient secretions in the channel contained ATP but not much protein.

Obee et al[8] have recommended that 500 RLUs be the benchmark for appropriate cleaning within endoscope channels when using the Biotrace ATP kits (Biotrace International, Bridgend, UK) for liquid samples. However, they did not validate this benchmark or the sampling method used. Aiken et al[10] have shown that the detection limit varies for kits from different manufacturers. The benchmark selected may vary depending on the ATP kit manufacturer[7-11] and the application, but it should be based on validation studies that demonstrate what is routinely achievable in both simulated-use and clinical studies using a clearly defined sample collection protocol. Our study is the first to validate an ATP test kit

as a means of rapidly monitoring the cleaning adequacy of patient-used flexible endoscopes prior to HLD.

The ATP tests used for monitoring cleaning are not capable of reliably detecting low levels of microorganisms.[7,10,13] As such, ATP testing just before the patient procedure is of limited value because it would only detect ATP if there was adequate microbial replication (eg, in the current study, the detection of >200 RLUs in the L4 channel of a fully reprocessed endoscope stored for 3 days may reflect wet storage that allowed microbial replication to a level that was detectable by the ATP test). Furthermore, Turner et al[7] reported that disinfectants may inactivate ATP, and, therefore, we would recommend that using an ATP test post-HLD to monitor cleaning efficacy is not ideal. We recommend that the ATP test be used after manual cleaning as an audit tool to confirm adequacy of cleaning (ie, removal of both patient-derived organic materials and bioburden). Auditing this stage of endoscope reprocessing provides a rapid way to ensure that poorly cleaned endoscopes are reprocessed before proceeding to HLD and before they are used on the next patient.

Because the clinic's written protocol and the observational audit indicated good compliance with the endoscope manufacturer's channel cleaning instructions, it is not surprising that the overall channel cleaning compliance was 96%. However, it raises the following question: "Is 96% compliance with manual cleaning adequate?" Aumeran et al[5] have reported an outbreak of multi-resistant Klebsiella pneumonia after improper cleaning of an ERCP endoscope. Is it realistic to expect 100% cleaning compliance in busy endoscopy clinics? This could only be achieved if every endoscope channel was tested every time it was used. More data are needed to reliably answer these questions. It would also be valuable to use this ATP audit tool to compare a wide variety of endoscopy clinic settings where there may be varying levels of compliance with manual cleaning of channels. Specifically, it would be valuable to assess whether the ATP audit tool could detect when the brushing of endoscope channels had been omitted because Ofstead et al[4] showed that there was only 43% compliance with the brushing step. Furthermore, use of this type of audit tool for endoscopes reprocessed after-hours or for emergency procedures would be useful to ensure the cleaning has been adequate before HLD. Because reprocessing guidelines now require documentation of training and ongoing competency, use of this ATP audit tool

provides a constructive way to evaluate staff competency with respect to manual cleaning of endoscope channels.

The limitations of this study include (1) inability to confirm that the bedside flush was done on all endoscope channels tested, (2) inability to confirm that the expected flush volume per elevator guide-wire channel was achieved for the Endo-Flush pump, and (3) that not all endoscopes processed on a given day were tested by the ATP test.

In conclusion, our data demonstrate that, when following the endoscope manufacturer's manual cleaning instructions, 96% of channels for gastrointestinal endoscopes will achieve the validated benchmark of <200 RLUs for the ATP test kit evaluated. Further studies are needed to determine what level of cleaning compliance is necessary (eg, 96% vs 100%) and to determine the frequency of monitoring the cleaning process (eg, all endoscopes all the time vs some endoscopes each day).

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