

ORIGINAL ARTICLE

Effectiveness of adenosine triphosphate to monitor manual cleaning and disinfection efficacy of flexible endoscopes in Hong Kong

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Abstract

Background and Aim: Adenosine triphosphate (ATP) bioluminescence assay is widely adopted in the West to allow rapid evaluation of endoscopes for bacteriologic/biologic residue, but this practice is rarely adopted in Asia. In this continuous quality improvement program, we evaluated the utility of ATP in bacteriologic surveillance on endoscope reprocessing.

Methods: A total of 456 samples (304 ATP samples and 152 culture samples) of 38 flexible endoscopes were assessed after routine clinical use in a private hospital in Hong Kong. Endoscopes were assessed with an ATP system and bacterial cultures at different time points during the reprocessing.

Results: After pre-cleaning, the ATP values ranged from 228 to 65 163 relative light units (RLU) through all endoscope types. After manual cleaning, ATP values were decreased to 7–81 RLU (median, 19 RLU) for endoscope surface and 3–671 RLU (median, 12 RLU) for channel rinsate. There was a significant reduction in ATP levels between pre-cleaning and after manual cleaning. One of the 38 (2.6%) endoscopes (a duodenoscope) had an ATP value of 671 RLU from channel rinsate, which exceeded the benchmark for cleanliness of >200 RLU, and was sent back for re-cleaning. All endoscopes cultured no bacteria after high-level disinfection (HLD) by automated endoscope reprocessor (AER) and storage up to 24 h. ATP values were <200 RLU for all endoscopes after HLD and storage.

Conclusions: Adenosine triphosphate bioluminescence assay offers a rapid, practical, and cost-effective alternative for detection of endoscope microbial residue as well as a routine monitoring tool for endoscope cleanliness in the clinical setting.

Introduction

Endoscopic procedures are performed with complex, reusable, flexible instruments that may become heavily contaminated with biomaterial and microorganisms, including potential pathogens when inserted into patients' body.^{1,2} Infection with multidrug-resistant organisms has become a critical issue worldwide because of their associated increased morbidity, mortality, and financial burden on health care. Previous studies found that contaminated endoscopes have been linked to more outbreaks of healthcare-associated infections than any other medical devices.³ Several recent outbreaks of infection with carbapenem-resistant Enterobacteriaceae associated with contaminated duodenoscope overseas have forced a reassessment on the risk of cross-transmission related to endoscopic procedures, particularly endoscopic retrograde cholangiopancreatography (ERCP). These incidents have highlighted a need for endoscopy units to create a

surveillance program to ensure adequate high-level disinfection (HLD) of their endoscopes.⁴

Endoscope reprocessing by current, best evidence-based practice aims to provide a reusable endoscope that is safe for patient use.⁵ A few factors including lapses in reprocessing, bio-film formation, endoscope design, and endoscope damage have contributed to culture-positive microorganisms on endoscopes as well as associated infection.^{3,6} Methods of improving endoscope reprocessing, screening for contamination, and evaluating endoscope damage may be vital to prevent future infections and outbreaks.

Bacterial culture has been the standard tool to evaluate bacterial contamination, but it is labor-intensive and time-consuming as it takes a few days to inoculate the microorganisms. It is not a feasible solution to provide instant feedback before applying the endoscope to subsequent procedures and cannot be used to prevent outbreaks. On the other hand, adenosine

triphosphate (ATP) bioluminescence technology was widely adopted to evaluate the cleanliness of flexible endoscopes after manual cleaning in the United States and European countries for over 10 years,⁷ but such application is rarely adopted in Hong Kong and Asian countries.

There is a need for a rapid surveillance method to proactively monitor the compliance of flexible endoscope reprocessing. Testing for ATP bioluminescence, measured as relative light units (RLU), offers a practical, rapid, and low-cost approach. ATP is present in microorganisms as well as human cells, and its presence indicates microbial/biological residue in endoscopes. A study performed by Alfa *et al.*⁸ suggested that ATP bioluminescence of <200 RLU after completion of all manual cleaning steps was correlated with acceptable residual bioburden benchmarks, which would allow for effective subsequent HLD.⁹

In Union Hospital, Hong Kong, 15 128 endoscopic procedures were performed in 2021, highlighting the vital importance of endoscope reprocessing in our busy endoscopy unit.¹⁰ In 2019 and 2020, 4.47% and 5.38% of microbiological tests were found to be culture-positive in Union Hospital as compared with 12–24% in Western reports, respectively.¹¹ A series of improvement plans were made to address the issue of bacterial contamination of reprocessed endoscopes, including (i) improving the reprocessing cleanliness level of biopsy channel valves; (ii) changing gloves when handling the flexible endoscopes after manual cleaning to prevent cross contamination; (iii) competency training and audit of reprocessing staff on manual cleaning of endoscopes; (iv) competency training on culture sampling and ATP measurement of flexible endoscopes was initiated; and (v) environmental enhancement with continuous monitoring of temperature and relative humidity in the endoscope storeroom.

Union Hospital is regularly carrying out continuous quality improvement (CQI) programs to improve the quality of clinical services. This project is a CQI program aimed to evaluate the utility of ATP bioluminescence as an alternative method for surveillance of flexible endoscopes before and after the HLD process and storage as compared with bacterial culture.

Methods

Study design. This CQI project was performed at the Endoscopy and Day Surgery Center of Union Hospital, Hong Kong in 10 months between 29 January 2021 and 28 October 2021. Data from the usage of 38 flexible endoscopes, including Gastroscope model GIF-HQ290; Colonoscope model CF-HQ290L; Duodenoscope model TJF-260V; Bronchoscope model BF-26 and Cystoscope model CYF-240A (Olympus Medical Systems, Tokyo, Japan) were analyzed. These 5 models are used for approximately 80% of all endoscopic procedures in the Center. Consecutive patient-used endoscopes were tested for ATP bioluminescence at pre-cleaning stage, after manual cleaning, after HLD by automated endoscope reprocessor (AER), and at 4 h or 24 h of storage, while bacterial culture was performed on endoscopes after HLD by AER and at 4 h or 24 h of storage. The aim for applying ATP application after pre-cleaning in this study was to collect a baseline data to calculate the log reduction in different stages of reprocessing of flexible endoscopes in our hospital.

During this study, a standard seven-step procedure was performed in endoscope reprocessing, including (i) Pre-cleaning to prevent the formation of any biofilm immediately after

removing the endoscope from the patient; (ii) Performing Leak-Test to detect any damage to the external surface and internal channels of the scope; (iii) Manual Brushing and Flushing to flush any residual matter that may be lingering in the channels and ports; (iv) Rinsing and Drying to rinse the detergent from the endoscope with clean water; (v) Disinfection following the manufacturer's instructions with the AER^{12,13}; (vi) Flushing with Alcohol to promote drying of the channels and prevent bacterial growth; and (vii) Storage in an uncoiled, vertical position in a well-ventilated, clean, tidy, and dust-free environment with standard humidity at <75% and room temperature of 20–22°C.^{5,13} The entire process was well documented and recorded.

Bacterial sampling. Endoscope testing was performed in a room adjacent to the procedure room, which allowed rapid sampling and testing. Barrier separation between procedural, reprocessing, data collection, and testing activities could minimize the risk of environmental cross-contamination. Besides, disinfectant wipes on surfaces and restricting room access could ensure aseptic environmental conditions during data collection. Sampling staff was required to wear sterile gloves, gowns, and surgical masks with splash protection. To prevent contamination, gloves were changed between sampling and gowns were changed between endoscope encounters.

ATP bioluminescence. Patient-used endoscopes were tested for ATP bioluminescence at pre-cleaning stage, after manual cleaning, after HLD by AER, and at 4 h or 24 h of storage. ATP has been validated for assessing endoscope contamination.¹⁴ ATP values were tested using 3 M Clean-Trace Surface ATP and Clean-Trace Water ATP tests. A luminometer quantified ATP expressed in relative light units (RLU). In accordance with a validated benchmark for the cleanliness of endoscopes, a cutoff of 200 RLU^{8,9} was applied to evaluate external surface and channels of endoscopes before HLD.¹⁵

A 3 M Clean-Trace Surface ATP UXL-100 ATP surface test device measured ATP on the exterior surface of the endoscope. A swab sampling was obtained from the surface area extended from the tip to the 20-cm proximal mark on the insertion tube, using a single swiping movement from the mark toward the tip. Any ATP collected on the swab was measured with a 3 M Clean-Trace Luminometer UNG3.

To sample at endoscope suction–biopsy channels, the flush-only method was used. Exactly 40 ml of sterile water followed by 60 ml of air was flushed from the umbilical end to the distal tip of the endoscopes.⁸ Then the above steps were repeated in the four intervals of sampling. As per protocol, endoscopes with any failed ATP results after manual cleaning were sent back for an additional reprocessing cycle and repeat testing.⁸

Microbiological surveillance test (MST). Bacterial culture was performed from reprocessed endoscopes (after drying) from the distal end and instrument channel.^{12,16,17} Biopsy channel brush and the biopsy channel irrigation water were collected after HLD by AER and at 4 h or 24 h of storage. Specimens were sent to the laboratory for sterility tests, which needed 4 days of incubation time. Every bacterial growth was considered microbiological positive regardless of species or number of colony forming unit (cfu).

Statistical analysis. Data were entered into Excel (Microsoft Office, Microsoft Corporation, Redmond, WA, USA). Data were summarized with absolute and relative (percentages) frequencies, with median and range of ATP values at different encounters of endoscope surface and channel rinsate. Statistical comparison for quantitative variables was performed with Wilcoxon paired test. A *P*-value <0.05 was considered statistically significant.

Regarding both the sampling points (namely, endoscope surfaces and channel rinsate samples) of each flexible endoscope, the ATP value of the contamination level was detected at different encounters (i.e., pre-cleaning stage, after manual cleaning, after HLD by AER and at 4 h or 24 h of storage). At the end of each step throughout the entire cleaning process, *P*-values and logarithmic reduction (Log *R*) of the contamination were calculated.

Results

All reprocessing steps were performed in accordance with the standard operation procedure of the Center. Overall, 38 flexible endoscopes were sampled on two points at each encounter on endoscope surface and channel rinsate at pre-cleaning stage, after manual cleaning, after HLD by AER, and at 4 h or 24 h of storage. A total of 304 swabs were collected. ATP values from endoscope surface and endoscope channel rinsate at different encounters are listed in Table 1.

After pre-cleaning, ATP bioluminescence was performed on all 38 flexible endoscopes (Table 1); 100% (38 of 38) endoscopes were positive for contamination. Both endoscope surface and channel rinsate had high ATP levels, ranged from 228 RLU to 65 163 RLU (median, 2322 RLU and 1720 RLU, respectively) through all endoscope types (Table 1). Bacterial culture was not performed at this encounter.

After manual cleaning, ATP values ranged 7–81 RLU (median, 19 RLU) for endoscope surface and 3–671 RLU (median, 12 RLU) for endoscope channel rinsate. One of the 38 (2.6%) endoscopes (a duodenoscope) had an ATP value of 671 RLU from channel rinsate, which exceeded the benchmark for cleanliness of >200 RLU, was sent back for additional cleaning and retesting (Table 1). The ATP value of the channel rinsate was reduced to 132 RLU after the second round of manual cleansing. Bacterial culture was also not performed at this encounter.

After HLD by AER, 0% (0/38) of endoscope surface and channel rinsate was positive for contamination (Table 1). All endoscopes cultured no bacteria after HLD by AER. ATP values after HLD ranged from 2 RLU to 109 RLU (endoscope surface or endoscope channel rinsate) (Table 1).

Ten endoscopes had ATP value tested after 4-h storage and the remaining 28 endoscopes after 24 h of storage. None of the 38 endoscopes (0%) had contamination for endoscope surface and channel rinsate (Table 1). Endoscope surface had ATP values ranged 5–21 RLU (median, 9 RLU) and channel rinsate ranged 2–11 RLU (median, 3.5 RLU) after 4 h of storage. After 24 h of storage, the ATP values of endoscope surface ranged 3–138 RLU (median, 10.5 RLU) and channel rinsate ranged 3–81 RLU (median, 10 RLU) on the remaining 28 endoscopes, which were numerically higher than that after 4 h storage, but the

difference did not reach statistical significance. Bacteria were not detected from biopsy channel brush and biopsy channel irrigation water for all endoscopes.

There was significant reduction in ATP values of endoscope surfaces and channel rinsate samples from pre-cleaning to after manual cleaning (Table 2). Similarly, there were also significant reduction in ATP values of endoscope surfaces and channel rinsate samples from after manual cleaning to after HLD by AER. After storage, there were no significant differences in ATP values for the endoscope surfaces and the channel rinsate samples between after HLD by AER and after 4 h or 24 h of storage respectively. This reflects that the manual cleaning is effective and adequate before HLD, and storage for 4 h–24 h will not raise the ATP values significantly.

Discussion

In this CQI project, we have demonstrated a 0% microbiological contamination after HLD by AER and storage for our tested endoscopes. The number of contaminated endoscopes in our study is much lower than that reported by Moses and Lee,¹¹ who found 12%–24% positive cultures during a 10-year study period. To our knowledge, there had been no prior local study conducted to explore the utility of ATP to evaluate the cleanliness of flexible endoscopes after manual cleaning and/or after HLD by AER. In this project, we have validated the use of ATP as an alternative to bacterial culture for surveillance of microbiological contamination after cleaning.

One critical challenge of flexible endoscope reprocessing methods is high contamination on endoscopes after use. In general, the ATP values in channel rinsate samples at pre-cleaning and after manual cleaning tend to be higher than that of the endoscope surfaces (Table 2) due to the complex design of the devices and potential biofilm formation. This difficulty in cleaning the elevator mechanism/channel has been cited as the main factor contributing to transmission of infection by duodenoscopes.^{4,18} Nonetheless, there is nonexistent margin of safety to prevent cross-infection related to contamination of endoscopes. In the Endoscopy and Day Surgery Center of Union Hospital, endoscope reprocessing is performed under strict standard guidelines. We have shown a >4 log reduction in ATP value from pre-cleaning to after manual cleaning in all endoscopes on endoscope surfaces and channel rinsate samples, and an additional >2 log reduction in ATP value after HLD by AER. According to Rutala and Weber, cleaning (2–6 log reduction) and HLD (4–6 log reduction) are essential for patient safe instrument.¹⁹

One advantage of using ATP as the surveillance tool over bacterial culture is the availability of immediate feedback of results, so that staff can decide on the need of retesting or repeating the cleaning procedure. It is vital to ensure all endoscopes have adequate cleaning (ATP <200 RLU) before the HLD process because any remaining organic and inorganic residues may interfere with the subsequent disinfection process, which increases the risk of reprocessing failure and cross-infection in patients.^{5,13,15} Bacterial culture results are available only after a few days and cannot prevent any potential outbreak. In this study, the ATP result of one duodenoscope had high ATP value from channel rinsate sample (671 RLU) after the initial manual

TABLE 1 Endoscope surface and endoscope channel rinsate contamination by endoscope and sampling time (*n* = 38)

Endoscope identifier by type	Pre-cleaning			Manual cleaning			HLD by AER†			Storage ≤4 h†			Storage ≤24 h†		
	Endoscope surface ATP (RLU)	Channel rinsate ATP (RLU)	Channel rinsate ATP (RLU)	Endoscope surface ATP (RLU)	Channel rinsate ATP (RLU)	Channel rinsate ATP (RLU)	Endoscope surface ATP (RLU)	Channel rinsate ATP (RLU)	Channel rinsate ATP (RLU)	Endoscope surface ATP (RLU)	Channel rinsate ATP (RLU)	Channel rinsate ATP (RLU)	Endoscope surface ATP (RLU)	Channel rinsate ATP (RLU)	Channel rinsate ATP (RLU)
Gastroscopes (<i>n</i> = 13)															
G12	4277	1331	10	31	6	21	NA	NA	NA	6	4	NA	20	10	4
G13	23 638	3206	135	32	8	31	NA	NA	NA	8	10	NA	51	46	10
G14	5299	4758	47	19	18	109	NA	NA	NA	18	74	NA	34	29	46
G15	2518	65 163	33	19	26	9	NA	NA	NA	26	12	NA	10	10	74
G16	2926	2961	81	11	18	9	NA	NA	NA	18	3	NA	3	12	29
G17	4856	3847	7	25	40	12	NA	NA	NA	40	10	NA	138	10	12
G18	6139	6354	18	52	10	10	NA	NA	NA	10	25	NA	10	25	10
G19	3470	4951	12	36	23	75	NA	NA	NA	23	7	NA	120	7	25
G20	35 773	7962	10	18	23	24	NA	NA	NA	23	7	NA	11	56	7
G21	4118	1218	9	21	35	12	NA	NA	NA	35	30	NA	104	30	56
G22	1187	667	30	37	15	23	NA	NA	NA	15	81	NA	88	81	30
G23	8209	1060	25	80	33	18	NA	NA	NA	33	6	NA	12	6	81
G24	7119	2829	16	81	43	14	NA	NA	NA	43	0 (0/13)	NA	0 (0/13)	0 (0/13)	6
Percent positive for contamination (no./total)															
100 (13/13)															
Colonoscopes (<i>n</i> = 15)															
C12	339	874	11	24	14	8	NA	NA	NA	14	10	NA	20	10	10
C13	598	631	5	19	20	7	NA	NA	NA	20	3	NA	5	3	3
C14	583	781	6	14	34	38	NA	NA	NA	34	11	NA	17	11	11
C15	3202	9616	22	18	19	11	NA	NA	NA	19	28	NA	10	28	28
C16	5317	9079	35	18	16	6	NA	NA	NA	16	9	NA	9	9	9
C17	2632	1342	16	27	20	10	NA	NA	NA	20	6	NA	11	6	6
C18	2126	3394	3	7	5	2	NA	NA	NA	5	7	NA	4	7	7
C19	381	426	37	25	23	6	NA	NA	NA	23	11	NA	10	11	11
C20	8757	5180	3	15	23	37	NA	NA	NA	23	5	NA	30	5	5
C21	336	587	12	7	17	9	NA	NA	NA	17	4	NA	6	4	4
C22	605	789	8	19	11	18	NA	NA	NA	11	4	NA	7	4	4
C23	1543	2097	13	23	21	4	NA	NA	NA	21	11	NA	10	11	11
C24	438	365	9	23	16	5	NA	NA	NA	16	6	NA	15	6	6
C25	531	425	9	9	25	9	NA	NA	NA	25	9	NA	9	9	9
C26	1223	2186	27	9	17	13	NA	NA	NA	17	3	NA	7	3	3
Percent positive for contamination (no./total)															
100 (15/15)															
ERCP/duodenoscope (<i>n</i> = 2)															
D2	31 388	49 745	671 (132)*	50	61	19	21	5	NA	61	NA	NA	NA	NA	NA
D3	1471	1280	5	39	8	3	6	4	NA	8	NA	NA	NA	NA	NA
Percent positive for contamination (no./total)															
100 (2/2)															
Bronchoscopes (<i>n</i> = 3)															
B2	5139	8060	7	21	12	5	15	3	NA	12	NA	NA	NA	NA	NA

(Continues)

TABLE 1 (Continued)

Endoscope identifier by type	Pre-cleaning		Manual cleaning		HLD by AER [†]		Storage ≤4 h [†]		Storage ≤24 h [†]	
	Endoscope surface ATP (RLU)	Channel rinse ATP (RLU)	Endoscope surface ATP (RLU)	Channel rinse ATP (RLU)	Endoscope surface ATP (RLU)	Channel rinse ATP (RLU)	Endoscope surface ATP (RLU)	Channel rinse ATP (RLU)	Endoscope surface ATP (RLU)	Channel rinse ATP (RLU)
B4	1183	2178	34	9	21	16	5	2	NA	NA
B5	3205	6252	18	12	15	4	9	2	NA	NA
Percent positive for contamination (no./total)	100 (3/3)	100 (3/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	NA	NA
Cystoscopes (n = 5)										
U1	655	956	11	4	14	7	8	2	NA	NA
U3	528	228	16	7	21	7	9	5	NA	NA
U4	886	607	12	5	22	8	9	4	NA	NA
U5	446	514	13	23	19	10	15	11	NA	NA
U6	498	303	13	21	22	14	6	6	NA	NA
Percent positive for contamination (no./total)	100 (5/5)	100 (5/5)	0 (0/5)	0 (0/5)	0 (0/5)	0 (0/5)	0 (0/5)	0 (0/5)	NA	NA

[†]Second test.

[‡]Bacterial culture was performed at these sampling times.

AER, automated endoscope reprocessor; ATP, adenosine triphosphate; HLD, high-level disinfection; NA, not applicable; RLU, relative light units.

TABLE 2 Median (range) ATP values and log reduction in ATP values of endoscopes at different encounters (n = 304)

Area tested	(A) Pre-cleaning ATP (RLU)	(B) After manual cleaning ATP (RLU)	P values (A – B)	Log reduction (A – B)	(C) After HLD by AER ATP (RLU)	P values (B – C)	Log reduction (B – C)	(D) Post-AER's cleaning and disinfection at 4 h storage ATP (RLU)	P values (C – D)
Endoscope surfaces	2322 (336–35 773)	19 (7–81)	<0.05	4.1	19.5 (5–61)	<0.05	2.0	9 (5–21)	10.5 (3–138)
Channel rinse samples	1720 (228–65 163)	12 (3–671)	<0.05	4.2	10 (2–109)	<0.05	2.1	3.5 (2–11)	10 (3–81)

Log reduction = $\log_{10}(A) - \log_{10}(B)$. Percent reduction = $[(A - B) \times 100] / A$. Where: A is the no. of viable microorganisms before treatment; B is the no. of viable microorganisms after treatment. AER, automated endoscope reprocessor; ATP, adenosine triphosphate; HLD, high-level disinfection; RLU, relative light units.

cleaning, and it was sent back for additional cleaning.²⁰ The immediate feedback also raised the alertness of clinical staff and facilitated the planning to enhance staff training and the reprocessing procedure of flexible endoscopes.

Temperature and humidity are two environmental factors for bacterial growth on flexible endoscopes.¹⁰ In the Endoscopy and Day Surgery Center, we have installed temperature and humidity meters at the storage room for continuous monitoring and recording of storage condition according to the Society of Gastroenterology Nurses and Associates (SGNA) Standard (Temperature 20–22°C; Humidity <75%).⁵ In this study, we have obtained “Pass” on ATP surveillance (<200 RLU) and no bacterial growth on culture on all flexible endoscopes after HLD by AER and at 4 h or 24 h of storage from endoscopy surfaces and channel rinsate samples.

There are a few limitations in this study. The sample size of 38 endoscopes is relatively small. But our results from multistaged surveillance by ATP and culture are reassuring. Second, it has been reported by others that ATP detection technology could have different reporting limit for Gram-negative *versus* Gram-positive bacteria, which might lead to false-negative result in Gram-negative-bacteria-contaminated sample.^{21,22} It might be due to incomplete cell lysis and release of ATP molecules from Gram-negative bacteria.²¹ Previous reports demonstrating this observation used different systems other than the one we used in this project.^{21,22} As we did not discover any culture-positive sample in our study, we were not able to assess whether the same observation could be reproducible in the detection system we used.

Nevertheless, bacterial culture is by no means replaceable by ATP testing at this moment. The correlation of ATP level with bacterial load is still uncertain; an ATP value of <200 RLU may not indicate sterility of the endoscope. Furthermore, keeping bacterial culture as a routine checking to detect the trend and type of any organisms growing is essential in preventing future potential endoscope outbreaks.

In conclusion, ATP bioluminescence assay is proven to be practical and effective in the local private hospital setting as a surveillance tool for endoscope cleaning. The most critical and cost-effective testing stage would be after manual cleaning to timely inform the need of additional cleaning of the endoscope, as it is highly unlikely one will get a positive result after HLD. The use of ATP surveillance for endoscopy cleanliness has been proven useful in other regional endoscopy unit.²³ Together with stringent protocols of endoscope reprocessing, endoscopy centers can prevent future endoscope-associated infections and outbreaks. Recently, the updated Association for the Advancement of Medical Instrumentation (AAMI ST91) guideline for “flexible and semi-rigid endoscope processing in health care facilities” has also included ATP as one of the markers useful for user verification and benchmarking of the cleaning processes.²⁴ This study supports the recommendation to use ATP to monitor the effectiveness of manual cleaning and disinfection of flexible endoscopes in Hong Kong.

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