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Major article

Comparison of adenosine triphosphate, microbiological load, and residual protein as indicators for assessing the cleanliness of flexible gastrointestinal endoscopes

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Background: This study evaluated 3 potential indicators of gastrointestinal endoscope cleanliness: adenosine triphosphate (ATP), microbiological load, and protein.
Methods: Before and after cleaning, ATP and microbiological load were determined from swabs of exterior surfaces and rinses of interior suction/accessory channels. Similarly, before and after cleaning, residual protein was determined from rinses of interior suction/accessory channels.
Results: Before cleaning, ATP values were 10,417 relative light units (RLU) from the exterior endoscope surface and 30,281 RLU from the suction/accessory channel rinsates. After cleaning, these ATP values were decreased to 82 RLUs and 104 RLUs, a statistically significant difference. A similar trend was observed with microbiological load, but the change in residual protein from before cleaning to after cleaning was not significant. ATP values reliably reflected microbiological colony counts.
Conclusions: ATP measurement can provide a reliable, rapid and practical assessment of endoscope cleanliness for routine monitoring in the clinical setting.

Gastrointestinal endoscopes have been used widely for the diagnosis and examination of gastric cancer, gastric ulcer, duodenal ulcers, and other gastrointestinal conditions in hospitals worldwide. In the Osaka University Hospital, approximately 7,000 endoscopies are performed for diagnosis, examination, and various treatments each year.

After an endoscopic procedure, the external surfaces and the interior suction and accessory channels of the endoscope are contaminated with various infectious biological fluids, such as blood, as well as minute tissue fragments. To prevent cross-infection with these contaminants, guidelines have been issued in various countries for cleaning and disinfection of contaminated endoscopes.[1-5] In Japan, various reprocessing methods, are recommended, including manual cleaning of the endoscope surface in an enzymatic detergent with a sponge and brushing the interior of the suction and accessory channels, in accordance with guidelines issued in other countries.

Cleaning and disinfecting the surface and interior lumen of an endoscope to the degree required is not easy, however. Contamination of the air and water channel segments of endoscopes that cannot be reached with brushes given the complex structure of the endoscope have been reported,[6] as have cases of hepatitis C virus (HCV) transmission attributed to poor cleaning and disinfection of endoscopes.[7] Detailed reviews of bacterial infections and common sites of contamination in flexible upper and lower gastrointestinal endoscopes (investigated between 1974 and 1991) and flexible bronchoscopes (investigated between 1975 and 1989), have been published.[8]

Guidelines have been issued on the allowable amount of remaining protein per surgical instrument (eg, scissors, forceps, tweezers) after cleaning.[9] However, the currently available guidelines offer no clearly defined indicators for determining the contamination level and for assessing the cleanliness of endoscopes. One study provided a detailed determination of adenosine triphosphate (ATP) and microorganism levels detected before and after cleaning of several endoscopes.[10] ATP, microorganism load, and

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protein also have been investigated as potential indicators of the cleanliness of flat surfaces in a food processing setting,[11] but there seems to be no established consensus on the appropriateness and thresholds of these 3 indicators for assessing endoscope cleanliness.

In the present study, we measured the amounts of ATP and microorganisms present on the external surfaces of endoscopes, as well as the levels of ATP, microorganisms, and protein present in the rinsate of the suction and accessory channels, before and after manual cleaning of endoscopes immediately after their removal from patients. The aim was to determine the most appropriate and practically useful indicator for characterizing the contamination level and cleanliness status of endoscopes in routine clinical settings.

MATERIALS AND METHODS

Gastrointestinal endoscopes

A total of 12 gastrointestinal endoscopes (models GIF-XQ260, GIF-Q260, GIF-H260, GIF-Q240Z, GIF-H260, and GIF-H260Z; Olympus Medical Systems, Tokyo, Japan) used in 41 patients at Osaka University Hospital were subjected to analysis. These 6 models are used for approximately 80% of the diagnostic, examination, and various treatment procedures involving endoscopes at Osaka University Hospital. In the usual cleaning practice, 200 mL of 1% diluted enzymatic detergent is aspirated into the suction and accessory channels of the endoscope immediately after it is removed from the patient. This detergent aspiration procedure was omitted during the study period (August 25-28, 2009), however, to eliminate possible interference from the chemical components of the detergent (eg, surfactants) when determining the concentration of ATP and establishing aerobic bacterial colony counts.

Leak testing and manual cleaning of endoscopes

In this procedure, a plastic tray set was filled with tap water in a washing sink, and the used endoscope was immersed in the tray. A leak detector (MU-1; Olympus Medical Systems, Tokyo, Japan) was connected to the ventilation hole and the absence of pinholes was confirmed under continuous air infusion by the MU-1 leak detector. The manual cleaning protocol at Osaka University Hospital is as follows:

1. The endoscope is immersed in 1% 3M Rapid Multi-Enzyme Cleaner 70500-D (3M, St Paul, MN) detergent in a thermostatic bath kept at 40°C.
2. Using a 20-mL plastic syringe, enzymatic detergent is flushed into the suction port and instrument channel port. This process is repeated, and the scope is then immersed in the thermostatic bath and soaked for 5 minutes at 40°C.
3. During immersion, the suction valves, biopsy valves, and any accessories are cleaned with a sponge.
4. After a 5-minute immersion, the exterior surface of the scope is cleaned with a sponge, and the interior surface of the instrument channel port is cleaned with a brush (BW-20T; Olympus Medical Systems) 3 times while in the thermostatic bath.
5. A screw brush (DISPO CLEAN C; Normandie Endo Technologie, Grentheville, France) is drawn through the instrument channel port and pulled out from the distal end of the port while in the thermostatic bath.

In routine reprocessing, the endoscope is placed in an automated reprocessor (OER-2; Olympus Medical Systems) after manual cleaning for further cleaning and high-level disinfection. In this study, however, endoscope cleanliness was assessed immediately after the manual cleaning protocol without automated reprocessing.

Sampling from the exterior endoscope surface before and after cleaning, and measurement of ATP and microorganism load

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

Similarly, to determine microbial colony counts, swab samples from the exterior surface of the endoscope were obtained with a 3M Quick Swab as described above and inoculated onto a 3M Petrifilm Aerobic Count Plate. After incubation for 24 hours at 37°C, the numbers of colonies were counted using a 3M Petrifilm Plate Reader.

Processing rinsates obtained from the suction and accessory channels before and after cleaning, and measuring ATP, microorganism load, and protein in these rinsates

A 10-mL aliquot of distilled water was infused rapidly with a syringe into the channel of the endoscope being examined, and the liquid discharged from the tip of the endoscope was collected in a beaker. Then 10 mL of air was infused with a syringe into the channel to evacuate any liquid remaining in the channel into the beaker. The liquid in the beaker was aspirated with the syringe and rapidly reinfused into the channel. Based on our previous finding that 5 infusions were sufficient to thoroughly remove contaminants, this procedure was repeated 5 times, and the final liquid volume in the beaker served as the channel rinsate sample for analysis.

The 3M Clean-Trace Water Total ATP AQT 100 water test device, along with the 3M Clean-Trace Luminometer UNG 3, were used to measure ATP in the rinsate samples. To determine colony counts, a 1-mL aliquot of each channel rinsate specimen was inoculated onto a 3M Petrifilm Aerobic Count Plate. The channel rinsate specimen obtained before cleaning was diluted by 100 times or 1,000 times with distilled water to obtain 30-300 colonies per plate. The channel rinsate specimen obtained after cleaning was not diluted. The number of colonies growing after 24 hours of incubation at 37°C was counted using a 3M Petrifilm Plate Reader.

To characterize the amount of residual protein, 1 mL of the channel rinsate specimen was mixed with 3 mL of Coomassie protein assay reagent (Thermo Fisher Scientific, Rockford, IL) and, after allowing the reaction to occur at room temperature for 20 min, the absorbance at 595 nm was measured with a spectrophotometer (UV-2450; Shimadzu, Kyoto, Japan).[12]

RESULTS

Table 1 presents data (mean, maximum, and minimum values; standard deviation; and coefficient of variance) on ATP and microorganism load measured on the external endoscope surfaces, along with ATP, microorganisms, and protein analyzed in the channel rinsate specimens before and after cleaning. Although 41 endoscopes were subjected to these measurements, Table 1 shows the results for 32 endoscopes before cleaning and 35 endoscopes after cleaning, after the exclusion of 9 scopes that were contaminated with indigo carmine. Although 1% indigo carmine (Wako Pure Chemical Industries, Osaka, Japan) is often used in clinical practice, the presence of this dye turns the rinsate blue, which interferes with protein measurement. In addition, only 39 scopes were analyzed for ATP, and 37 scopes were analyzed for microorganisms, because in some cases the sampling procedure for this

Table 1
Measurement of ATP levels, microorganism colony counts, and protein levels on endoscope surfaces and in channel rinsate samples before and after cleaning (before cleaning/after cleaning)

	Endoscope surfaces*		Channel rinsate samples†		
	ATP, RLU/sample	Colony count, CFU/sample	ATP, RLU/sample	Colony count, CFU/sample	Protein, μg/sample
Cases assessed	39/39	37/37	41/41	41/41	32/35
Mean value	10,417/82	5,143/1	30,281/104	95,827/14	36/20
Maximum value	149,397/169	39,000/29	45,362/407	1,000,000/53	266/60
Minimum value	177/51	0/0	233/45	100/0	10/10
Standard deviation	24,402/21	9,053/5	72,761/57	174,369/15	57/16
Coefficient of variance	234/25	176/436	240/55	182/113	156/79

*The surface was wiped with a cotton swab using a single swiping movement from the 20-cm proximal marking to the tip of the insertion tube of the endoscope.
†A 10-mL aliquot of distilled water was infused rapidly into the suction and accessory channels, and the washing liquid was recovered from the endoscope tip into a beaker. This washing liquid was reinfused into the channels and recovered. This procedure was repeated 5 times.

study had to be cancelled in favor of routine cleaning and disinfection, to avoid delays in treatment.

On the external surface of the endoscope, the average ATP value before cleaning was 10,417 relative light units (RLU)/sample, and the corresponding average bacterial colony count was 5,143 colony-forming units (CFU)/sample. After cleaning, these respective average values were decreased to 82 RLU/sample and 1 CFU/sample. Before cleaning, the channel rinsate samples had an average ATP value of 30,281 RLU/sample, an average bacterial colony count of 95,827 CFU/sample, and an average residual protein concentration of 36 μg/sample. After cleaning, these corresponding values were 104 RLU/sample, 14 CFU/sample, and 20 μg/sample.

Figure 1 shows the changes in the levels of ATP, microorganisms, and protein measured in the channel rinsate specimens before and after cleaning. A paired *t* test for these samples yielded *P* values of .011 for ATP level, .001 for microorganism colony count determination, and .078 for protein level. The *P* values for the ATP level and colony count showed a statistically significant difference in both of these indicators from before cleaning to after cleaning.

Similar results were obtained for ATP levels and the colony counts on the endoscope surfaces before and after cleaning. A paired *t*-test yielded *P* values of .012 for ATP level and .001 for colony count on the endoscope surfaces, demonstrating a statistically significant difference in both of these indicators from before cleaning to after cleaning.

DISCUSSION

Gastrointestinal endoscopes are used for various examinations, procedures, and treatments related to lesions of the gastrointestinal tract, including cancer of the stomach, duodenum, and large or small bowel. The surfaces and channels of these endoscopes are often contaminated by blood, mucus, tissue, and organ contents (ie, contaminants) of human origin. Thus, components of blood, mucus, or tissue may serve as indicators of contamination level when assessing the cleanliness status of an endoscope.

ATP is present at high concentrations in both animals and plants, including microorganisms, and has previously been studied in the context of prolonging the storage shelf life of blood for transfusion,[13] as well as measurement of microorganisms[14] and several other applications. Swab-based assay kits using the ATP-driven luciferin/luciferase bioluminescent reaction to quantify the presence of ATP are now available, as are sensitive, easy-to-use handheld devices for measuring luminescence. These tools have now been widely used to evaluate contamination levels and confirm the cleanliness of environmental surfaces in food factories[15,16] and hospitals.[17,18] A patient room cleaning monitoring program using a value of $\leq$500 RLU of ATP to indicate a clean surface has been reported,[19] as has the use of ATP for assessing the cleanliness of surgical instruments.[20]

In this study, the levels of ATP and microorganisms present on the external surfaces of several endoscopes, as well as levels of ATP, microorganisms, and protein in channel rinsate samples obtained from the interior surfaces of the suction and accessory lumens, were measured to investigate their validity as indicators of the contamination level and cleanliness status of used endoscopes. Swabs obtained from the external surfaces of endoscopes using a swiping movement from the 20-cm mark to the tip of the scope were used to analyze ATP and microorganism levels. In a more rigorous evaluation, the entire surface of the scope should be wiped with a cotton swab, or, alternatively, a small amount of the distilled water (or physiological saline) used for rinsing the entire surface of the endoscope could be used as the sample. However, as a clinically practical and useful measurement method, we used the sampling procedure described above.

The mean ATP level determined from the exterior endoscope surfaces before cleaning was 10,417 RLU/sample (minimum, 177 RLU; maximum, 149,397 RLU), and the corresponding aerobic microorganism colony count was 5,143 CFU/sample (minimum, 0 CFU; maximum, 39,000 CFU). After cleaning, the mean ATP level decreased to 82 RLU/sample (minimum, 51 RLU; maximum, 169 RLU), and the mean colony count decreased to 1 CFU/sample (minimum, 0 CFU; maximum, 29 CFU). For the channel rinsates, the mean ATP level and colony count before cleaning were 30,281 RLU/sample (minimum, 233 RLU; maximum, 45,362 RLU) and 95,827 CFU/sample (minimum, 100 CFU; maximum, 1,000,000 CFU), respectively, and the corresponding values after cleaning decreased to only 104 RLU/sample (minimum, 45 RLU; maximum, 407 RLU) and 14 CFU/sample (minimum, 0 CFU; maximum, 53 CFU). Thus, there were clear and statistically significant differences in the amounts of both ATP and microorganisms on the scope surfaces and in the channel rinsates recorded before and after cleaning.

The mean residual protein level in the channel rinsate samples was 36 μg/sample (minimum, 10 μg; maximum, 266 μg) before cleaning and 20 μg/sample (minimum, 10 μg; maximum, 60 μg) after cleaning; this difference was not statistically significant. Detection of large amounts of proteins and endotoxins in the suction channels of bronchoscopes, duodenoscopes, and colonoscopes has been reported previously.[21] However, in the present study, because there were few cases of invasive operations as part of the endoscopic treatment, protein levels were low even before cleaning. Because not all uses of endoscopes involve invasive operations, the value of protein concentration as an indicator of endoscope contamination level appears to be less significant than that of ATP or microorganisms.

Among the 3 possible indicators investigated in this study, ATP and microorganisms proved to be useful indicators for assessing the contamination level and cleanliness status of the endoscopes. However, because determination of bacterial colony counts typically requires an incubation time of 24 hours, ATP is likely to be the

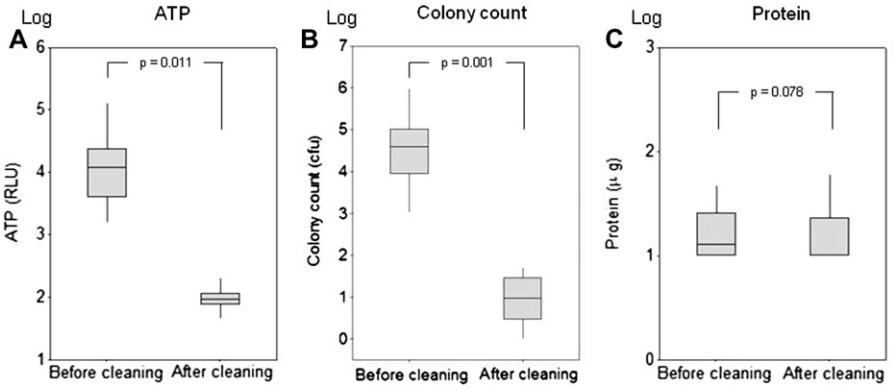

Fig 1. Interquartile range boxplot of changes in ATP levels (A), microorganism colony counts (B), and residual protein levels (C) in the channel rinsate samples before and after cleaning.

superior routine monitoring system for its practical, real-time and quantitative evaluation capabilities in clinical settings.

Although 500 RLU as measured by the 3M Clean-Trace ATP monitoring system has been adopted as a threshold value for environmental surface cleaning in some studies,[17-19] the optimum threshold value strongly depends on the sampling technique and the nature of the samples themselves. This threshold value is based on a 10 cm $\times$ 10 cm area of swabbing on a hospital environmental surface. Based on our measurements of ATP levels and microbial counts, as well as a review of current manual cleaning protocols, Osaka University Hospital has defined the following institutional criteria for allowable ATP levels remaining after manual cleaning: 169 RLU (maximum) for external endoscope surfaces and 407 RLU (maximum) for channel rinsate specimens. In addition, efforts are ongoing to reduce the value and variability of ATP measurements by routine ATP monitoring using this allowable value, educating Central Sterile Supply Department (CSSD) technicians, providing periodical process verification, and reviewing the cleaning protocol.

At present, to promote the routine cleaning monitoring system using ATP as an indicator, a training course on swabbing and preparation of channel rinsates is given to cleaning operators at the hospital. The CSSD reprocesses all of used endoscopes for diagnosis, examination, and various treatments at the Endoscopic Center, operating rooms, and Emergency Department. The CSSD is planning to routinely monitor endoscope cleaning using ATP for all endoscopes that are reprocessed; however, endoscopic procedures are most often performed in the morning, and thus for some endoscopes, monitoring of cleaning using ATP cannot be done for turnaround in the morning.

In this study, we set the cleaning temperature at 40°C to optimize the cleaning performance of the enzymatic detergent, and used screw brushes to provide better cleaning of the suction and accessory channels.[22] However, because manual cleaning of endoscopes and assessment using an ATP monitoring system vary among institutions, the appropriate threshold value of ATP used to assess endoscope cleanliness should be determined by each individual institution.

References

1. Alvarado CJ, Reichelderfer M. Association for Professionals in Infection Control. APIC guideline for infection prevention and control in flexible endoscopy. Am J Infect Control 2000;28:138-55.
2. American Society for Gastrointestinal Endoscopy. Multi-society guideline for reprocessing flexible gastrointestinal endoscopes. Gastrointest Endosc 2003; 58:1-8.
3. Heeg P. Reprocessing endoscopes: national recommendations with a special emphasis on cleaning: the German perspective. J Hosp Infect 2004;56(Suppl 2): S23-6.
4. Rutala WA, Weber DJ. Reprocessing endoscopes: United States perspective. J Hosp Infect 2004;56(Suppl 2):S27-39.
5. Darbord JC. Importance of cleaning for reprocessing endoscopes and thermolabile sterile medical devices: French use and regulations. J Hosp Infect 2004; 56(Suppl 2):S40-3.
6. Ishino Y, Ido K, Koiwai H, Sugano K. Pitfalls in endoscope reprocessing: brushing of air and water channels is mandatory for high-level disinfection. Gastrointest Endosc 2001;53:165-8.
7. Bronowicki JP, Venard V, Botté C, Monhoven N, Gastin I, Choné L, et al. Patient-to-patient transmission of hepatitis C virus during colonoscopy. N Engl J Med 1997;337:237-40.
8. Spach DH, Silverstein FE, Stamm WE. Transmission of infection by gastrointestinal endoscopy and bronchoscopy. Ann Intern Med 1993;118:117-28.
9. Roth K, Michels W. Inter-hospital trials to determine minimal cleaning performance according to the guideline by DGKH, DGSV and AKI. Zentr Steril 2005;13:106-16.
10. Obee PC, Griffith CJ, Cooper RA, Cooke RP, Bennion NE, Lewis M. Real-time monitoring in managing the decontamination of flexible gastrointestinal endoscopes. Am J Infect Control 2005;33:202-6.
11. Moore G, Griffith C, Fielding L. A comparison of traditional and recently developed methods for monitoring surface hygiene within the food industry: a laboratory study. Dairy Food Environ Sanit 2001;21:478-88.
12. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
13. Beutler E, Baluda MC. Simplified determination of blood adenosine triphosphate using the firefly system. Blood 1964;23:688-98.
14. Chappelle EW, Levin GV. Use of the firefly bioluminescent reaction for rapid detection and counting of bacteria. Biochem Med 1968;2:41-52.
15. Tebutt GM. Comparison of traditional and rapid methods for assessing the risk of bacterial cross-contamination from cutting boards. Int J Environ Health Res 1999;9:67-74.
16. Worsfold D, Griffith CJ. An assessment of cleaning regimes and standards in butchers' shops. Int J Environ Health Res 2001;11:245-56.
17. Malik RE, Cooper RA, Griffith CJ. Use of audit tools to evaluate the efficacy of cleaning systems in hospitals. Am J Infect Control 2003;31:181-7.
18. Boyce JM, Havill NL, Dumigan DG, Golebiewski M, Balogun O, Rizvani R. Monitoring the effectiveness of hospital cleaning practices by use of an adenosine triphosphate bioluminescence assay. Infect Control Hosp Epidemiol 2009;30:678-84.
19. Griffith CJ, Cooper RA, Gilmore J, Davies C, Lewis M. An evaluation of hospital cleaning regimes and standards. J Hosp Infect 2000;45:19-28.
20. Takashina M. Application of a bioluminescent method for checking cleaning results. Zentr Steril 2001;9:248-58.
21. Alfa MJ, Degagne P, Olson N. Worst-case soiling levels for patient-used flexible endoscopes before and after cleaning. Am J Infect Control 1999;27:392-401.
22. Charlton TS. A comparison of the efficacy of lumen-cleaning devices for flexible gastrointestinal endoscopes. Aust Infect Control 2007;12:78-83.