Original article

# Evaluation of the total adenylate (ATP + ADP + AMP) test for cleaning verification in healthcare settings

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Keywords

ATP + ADP + AMP • Hygiene monitoring • Healthcare settings • High touch surface • Endoscope

## Summary

**Introduction**. *Evaluation of cleaning methods is the first step in the prevention of healthcare-associated infections. ATP hygiene monitoring tests are widely used for assessing the effectiveness of cleaning procedures. The test is easy to use and gives immediate results, however, ATP can be metabolized and degraded to ADP and AMP. Recently, a total adenylate [ATP + ADP + AMP(A3)] monitoring test has been developed. Our objective was to evaluate the usefulness of the A3 test for cleaning verification in healthcare settings.*
**Methods**. *The detection sensitivities of the ATP and the A3 tests were compared using blood, and debris derived from gloved-hand method and endoscopes immediately after endoscopic examination. The performance of the A3 test in monitoring cleanliness of high touch surfaces in the hospital and endoscopes at each cleaning step was also evaluated.*
**Results**. *For the hemolysate, the measurement values of the A3 test were stable, although ATP was promptly degraded. In debris from hands, the amount of A3 was 20 times higher than that of ATP. The detection sensitivities of the A3 test on residues derived from gastroscopes and colonoscopes were 3 and 8 times higher, respectively, than those from the ATP test. A field study indicated that a large number of microorganisms tend to show high A3 values on high touch surfaces in the hospital and on endoscopes.*
**Conclusions**. *The A3 test showed higher detection sensitivities than the conventional ATP test for organic debris associated with healthcare settings.*

## Introduction

In the last decade, much effort has been focused on the prevention of healthcare-associated infections (HAI). There are reports that contaminated surfaces, surgical instruments, and endoscopes cause the transmission of hospital pathogens [1-3]. Cleaning, disinfection, and sterilization of environmental surfaces, instruments, and hands are fundamental steps in reducing their potential contribution to the incidence of HAI. It is widely accepted that thorough cleaning is a prerequisite to effective disinfection/sterilization. According to the guideline for disinfection and sterilization in healthcare facilities from the Centers for Disease Control and Prevention (CDC), organic material that remains on the surfaces could interfere with the disinfection procedures [4]. First, some organic matter can interfere with the antimicrobial activity of disinfectants [4, 5]. This interference occurs due to a chemical reaction between the germicide and the organic matter, resulting in a complex that is less germicidal, reducing the active germicide available for disinfection/sterilization or totally eliminating germicidal activity. In addition, the organic material can protect microorganisms from the germicide by acting as a physical barrier [4, 5]. Therefore, rapid and easy evaluation methods for cleanliness and cleaning methods are in demand for use in healthcare settings.

Traditionally, visual inspection was the method used to assess the level of cleanliness, but visual acuity of inspectors affects the results. Furthermore, some specific structures, such as the inner surfaces of endoscopes are difficult to inspect. Measuring viable bacterial counts (VBC), using agar plates, has also been used; however, it takes 24-48 h to obtain results. Determination of residual protein contamination is also a widely used method [6-8]. However, since it involves laborious measurement procedures, immediate results are impossible. DNA-based techniques, including real-time polymerase chain reaction (PCR) test is highly sensitive to detect specific bacteria [9], but it is laboratory-use test and a wide range of the biomass can't be detected. Though the adenosine triphosphate (ATP) swabbing tests are not feasible to assay the bacteria accurately or specify the bacteria, they have attracted a lot of attention because they enable on site, rapid verification of sanitation procedures for the prevention of HAI [7, 8, 10-12].

ATP is found in all living beings where they produce effects both by intracellular and extracellular mechanisms. Intracellular ATP is primarily utilized to drive energy-requiring processes such as active transport, cell motility and biosynthesis, whereas extracellular ATP is considered a powerful signaling molecule [13]. Therefore, ATP is a more versatile molecule than a supplier of energy in both prokaryotic and eukaryotic organisms, and the presence of ATP on surfaces indicates improper cleaning and the presence of contamination, including organic debris and bacteria. Although the existence of ATP does not always mean the presence of living cells, the high levels of ATP after insufficient washing generally repre-


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sent a higher bacteria hazard by comparison with no detectable ATP or low levels of ATP after complete washing. The latest recommendation from the Healthcare Infection Control Practices Advisory Committee and CDC concerning environmental control in healthcare facilities states a category II recommendation to disinfect and clean high-touch surfaces (eg. doorknobs, bed rails, light switches) on a more frequent basis than minimal-touch surfaces [14]. CDC listed the ATP test as one of the methods that can be employed to rapidly evaluate the effectiveness of environmental cleaning [15].

Conventional ATP test systems, however, have a limitation in that ATP is degraded to adenosine diphosphate (ADP) and adenosine monophosphate (AMP) by heat, acid/alkali, and enzymes [16, 17]. Because conventional ATP tests cannot detect these degradation products, they can miss insufficient cleaning and sterilization. Recently, a novel hygiene monitoring system to measure total the adenylate content [ATP + ADP + AMP, (A3)] was developed based on the luciferin-luciferase assay, with the combination of two enzymes, pyruvate kinase and pyruvate phosphate dikinase, which can convert ADP into ATP and AMP into ATP, respectively [17]. The newly developed A3 assay system afforded stable bioluminescence signals for ATP, ADP, and AMP, simultaneously. The evaluation of the A3 test for hygiene monitoring in healthcare settings has been demanded.

In this study, the amounts of ATP, ADP, and AMP in hemolysate, debris derived from gloved-hand method and endoscopes immediately after endoscopic examination, were assayed. Field tests for monitoring cleanliness of high touch surfaces in the hospital and endoscopes at each cleaning step were also carried out.

## Methods

### Reagents

Analytical grade ATP·2Na, ADP·K, and AMP·2Na were purchased from Oriental Yeast (Tokyo, Japan).

### Measurement of the ratios of ATP, ADP and AMP

The amount of ATP, ATP + AMP, and A3 were assayed using commercially available test kits, the LuciPac II/Lumitester C-110, LuciPac Pen/Lumitester PD-30, and LuciPac A3 Surface/Lumitester PD-30 (Kikkoman Biochemifa, Tokyo, Japan), based on the luciferin-luciferase assay [17]. The sample collection swabs and testing devices, which contain the reagent and the extraction buffer are integrated in these kits. After a swab was removed from the tube, the sample solution was pipetted onto the swabs. The swab stick was then returned to the main tube and inserted completely. The tube was shaken to mix the sample, extraction solution, and reagent thoroughly. The tube was then inserted immediately into the luminometer, and the resulting luminescence was measured. All measurements were performed at 23°C, and data were recorded electronically. The measurement output was relative light units (RLU). According to the calibration curve study using reagent-grade adenylates in a previous report, it was verified that the A3 test afforded equivalent linear curves for amounts of ATP, ADP, and AMP in double logarithmic charts. Moreover, a given amount of ATP gave almost the same RLU value in the ATP, ATP + AMP, and A3 tests [17]. Therefore, the ratios of the three adenylates in any sample could be estimated by comparing their RLU values. RLUs derived from ADP and AMP were calculated by the values of A3-(ATP + AMP) and (ATP + AMP)-ATP, respectively. The ratios of ATP + AMP and A3 to ATP could be expressed as relative values, with the values of ATP being normalized to 1. Then, the ratios of ATP:ADP:AMP could be calculated as 1:(A3/ATP)-[(ATP + AMP)/ATP]:[(ATP + AMP)/ATP]-1.

### Monitoring degradation of adenylates in hemolysate

Hemolysate was prepared by 10-fold dilution of blood (Nippon Bio-Test Laboratories, Saitama, Japan) with nuclease-free water and incubating at 35°C for 2 h. A 10 µl sample was applied onto the moistened swab with 100 µl nuclease-free water and ATP, ATP + AMP, and A3 tests were carried out over a time-course. The measurements were repeated 5 times, and the means were reported. The abundance ratios were expressed as relative values, normalized to RLU values before incubation, which were considered 100%.

### Measurement of adenylates in gloved-hand sample

The gloved-hand samples were prepared in the following method [18]. Powder-free, nitrile gum gloves (AS ONE, Osaka, Japan) were worn by the subjects (n = 10) on one hand for 3 h. Then, 5 ml of nuclease-free water was introduced into the glove and the liquid was recovered. A blank sample was also prepared, as described above, using unused gloves. The ATP, ATP + AMP, and A3 tests were carried out using 100 µl of each sample. The resulting RLUs were obtained by subtracting the blank values. (The blank RLUs of ATP, ATP + AMP, and A3 inside the gloves were 3, 41 and 51, respectively.) The measurements were repeated 3 times, and the means for individuals were found. The ratios of ATP + AMP and A3 to ATP for individuals were calculated, then their means were obtained.

### The inhibitory effects of disinfectant and cleaning agent on the A3 assay

Hydrogen peroxide (30%, Wako Pure Chemical, Osaka, Japan) was diluted with nuclease-free water to 3%. Enzyme-based immersion cleaning agent (Power Quick, Saraya, Osaka, Japan) was diluted 100-fold with nuclease-free water to prepare a 1.0% dilution according to manufacturer's instructions. Ten microliters of these solutions or water and 10 µL aliquots of $5 \times 10^{-7}$ M adenylate solutions were pipetted onto swabs moistened with 80 µL of water, then the measurements were carried out. The RLU were expressed as relative values, with RLU values without chemicals being considered 100%.

The experiment was repeated 5 times for each aliquot, and the means are reported.

### Environmental sampling and testing procedures in the hospital

High touch surfaces in the hospital [14] were swabbed using the swabs of LuciPac A3 Surface moistened with tap water before and after cleaning using moistened microfiber cleaning cloths (Toraysee™, Toray Industries, Tokyo, Japan). The items (n = 35) tested were telephone receivers (n = 2), PC mice (n = 2), desks (n = 2), carts [top boards (n = 2) and handles (n = 2)], stethoscopes (n = 2), blood pressure meter pumps (n = 2) in the nurses' station, door handles (n = 2), nurse call buttons (n = 2), bedside tables (n = 2), bed rails (n = 2), light switches (n = 2), refrigerator door handles (n = 2) in the hospital room, corridor handrails (n = 2), stretcher bed (n = 1), wheelchair handle (n = 1), cart handle in the ward (n = 1), infant incubators (n = 2) in the newborn nursery, door handles of the treatment room (n = 1) and the communication room (n = 1). Duplicate samples were different. These surfaces (10 cm x 10 cm areas) were sampled, using vertical and horizontal swabbing, carried out 10 times.

Each swab was immersed and washed in 5% glucose solution (1 ml, Otsuka Pharmaceutical Co., Tokyo, Japan) in 1.5 ml microcentrifuge tubes to prepare the analytical samples. Because the luciferase reaction is inhibited by salt [19], the 5% glucose solution without adenylates was used for protecting bacteria from osmotic pressure (data not shown). The samples were immediately cooled in a styrene foam box with a frozen gel pack and a lid. The blank sample was also prepared without surface swabbing. (The blank RLUs of ATP, ATP + AMP, and A3 were 3, 5, and 7 respectively.) The ATP, ATP + AMP, A3 tests and VBC were carried out using 100 µl of each sample. VBC was tested using tryptone soya agar (TSA) with incubation at 35°C for 1 day. The resulting RLU and colony-forming units (cfu) were multiplied by 10 because the swab can hold 100 µl liquid and the sample can be considered to be ca. 10-fold diluted with the 1 ml 5% glucose solution. The ratios of ATP + AMP and A3 to ATP for each point were calculated using the measurement values, and their means were obtained.

### Sampling for gastrointestinal endoscopy and testing procedures

A 400 mm long stem swab (with a 2.8 mm/3.2 mm diameter cotton bud, LuciSwab, Kikkoman Biochemifa, Tokyo, Japan) was moistened with 5% glucose solution and inserted into the inner lumen of gastroscopes (2.8 mm working channel, n = 19, Olympus, Tokyo, Japan) and colonoscopes (3.2 mm working channel, n = 6, Olympus, Tokyo, Japan) from the distal tip to as far as the length of the swab permitted. Then the swab was pulled up slowly and immersed and washed in 5% glucose solution (1 ml) in 1.5 ml microcentrifuge tubes to prepare the analytical samples. The samples were immediately cooled in a styrene foam box with a frozen gel pack and a lid. The blank sample was also prepared without swabbing. ATP, ATP + AMP, and A3 tests; Bradford protein assay (Standard: Bovine serum albumin, Thermo Scientific, Waltham, MA, USA); and VBC were carried out after 1) removal, 2) manual cleaning, and 3) automated reprocessing (before alcohol flushing, Endoclens-D or -S, Johnson & Johnson, New Brunswick, NZ, USA). VBC was tested using TSA with incubation at 35°C for 1 day under aerobic conditions. The ratios of ATP + AMP and A3 to ATP for each instrument, just after endoscopic examination, were calculated using the measurement values, and their means were obtained.

## Results

### Monitoring of the change of adenylates in hemolysate

The ATP, ATP + AMP, and A3 tests showed 21035, 30141, and 36825 RLU for the hemolysate that was prepared by 10-fold dilution of blood with water. The (ATP + AMP)/ATP and A3/ATP ratios were 1.4 and 1.7, respectively, and the ratio of ATP:ADP:AMP was 1:0.3:0.4 (Tab. I). In the fresh hemolysate, ATP was the major adenylate. Successively, time-dependent monitoring of adenylates after incubation of the sample at 35°C for 2 h was also performed (Fig. 1). The results of the ATP, ATP + AMP, and A3 tests were 3882, 34733, and 39314 RLU after 1 h, and 226, 33616, and 33996 RLU after 2 h, respectively. The ratios of (ATP + AMP)/ATP and A3/ATP after 2 h were 148.7 and 150.4, and the ratio of ATP:ADP:AMP was 1:1.7:147.7 (Tab. I). These results demonstrate that ATP was decreased to below 20% after 1 h and almost completely decomposed to AMP after 2 h. This indicates that ATP is promptly degraded to AMP in hemolysates and the conventional ATP method may miss contamination from blood. The risk of missing blood contamination is likely decreased by adopting the ATP + AMP method. However, the measurement value was unstable, i.e. the amount of ATP + AMP was temporarily reduced by 7% after 15 min and eventually to around 110% after 30 min (Fig. 1). Since ADP in the hemolysate is estimated to be 18% of the A3 before incubation (Tab. I), ATP and ADP were likely decomposed and accumulated as AMP, around 110% for ATP + AMP appears to be reasonable. On the other hand, A3 was stable within a range of ± 8% (Fig. 1). Therefore, the A3 test is an effective tool for the rapid verification of cleaning procedures for surgical instruments contaminated with blood.

### Evaluations of adenylate ratios in gloved-hand samples

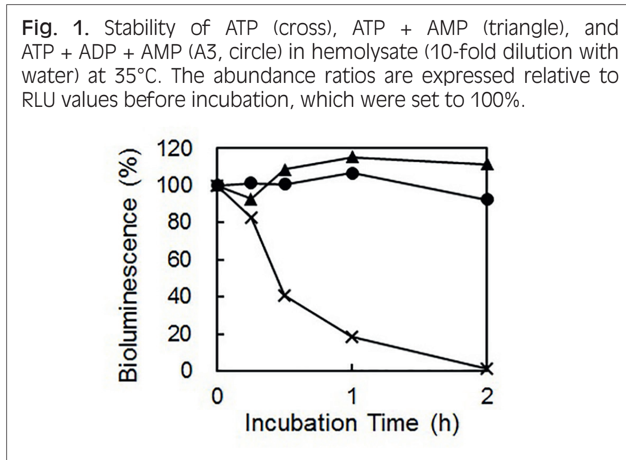
The RLUs of ATP, ATP + AMP, and A3 in gloved-hand samples (n = 10) were measured and the ratios of the results are shown in Table I. Gloved-hand samples generally contain sweat, skin cells, and bacteria. The means of (ATP + AMP)/ATP and A3/ATP were 8.2 and 20.1. The relative values of ADP and AMP were 11.8 and 7.2 fold higher than that of ATP. Since ATP was the minor

Tab. I. The means of ratios of ATP + AMP, ATP + ADP + AMP (A3), ADP, and AMP to ATP in hemolysate, sweat, and specimens from the hospital.

| Sample | ATP + AMP | A3 | ADP[a] | AMP[b] |
|---|---|---|---|---|
| Hemolysate (before incubation) | 1.4 | 1.7 | 0.3 | 0.4 |
| Hemolysate (after 1 h incubation) | 8.9 | 10.1 | 1.2 | 7.9 |
| Hemolysate (after 2 h incubation) | 148.7 | 150.4 | 1.7 | 147.7 |
| Gloved-hand samples | 8.2 | 20.1 | 11.8 | 7.2 |
| High touch surfaces | 4.3 | 7.5 | 3.2 | 3.3 |
| Debris from gastroscopes | 2.4 | 3.5 | 1.2 | 1.4 |
| Debris from colonoscopes | 4.9 | 7.6 | 2.7 | 3.9 |

The ratios of each adenylate to ATP are expressed relative to ATP, which was set to 1. [a]: A3-(ATP + AMP); [b]: (ATP + AMP)-ATP.

Fig. 1. Stability of ATP (cross), ATP + AMP (triangle), and ATP + ADP + AMP (A3, circle) in hemolysate (10-fold dilution with water) at 35°C. The abundance ratios are expressed relative to RLU values before incubation, which were set to 100%.




and ADP was the major adenylate in the gloved-hand samples, 20 and 2.4 times higher sensitivity in detection of debris from hands can be achieved by the A3 method, compared with the conventional ATP test and the ATP + AMP test, respectively. Since the A3 method seemed to be useful for the evaluation of environmental cleaning and hand washing, a field study for high touch surfaces in the hospital was carried out to evaluate the practical performance of the A3 test.

### The inhibitory effects of disinfectant and cleaning agent to the A3 assay

Hydrogen peroxide (3%) and commercially available enzyme-based immersion cleaning agent had little inhibitory effect on the A3 assays when they were added at 10% final volume (Tab. II).

### Field study for the measurements of adenylates and VBC on high touch surfaces in the hospital

The cleanliness of high touch surfaces (n = 35) in the nurse station, the hospital room, the ward, the newborn nursery, the treatment room, and the communication room before and after cleaning with moistened microfiber cleaning cloths were evaluated by adenylate assays and VBC. After the cleaning, the amounts of A3 decreased at all measurement points, and the mean rate of decline was 87%. For instance, A3 values and VBC reduced from 1280 RLU and 1670 cfu before cleaning to 100 RLU and 0 cfu after cleaning of the door handle of the patient's room. For the bed rail, 1100 RLU and 50 cfu before cleaning was improved to 160 RLU and 0 cfu after cleaning. The means of (ATP + AMP)/ATP and A3/ATP for all measurement points were 4.3 and 7.5, respectively. The relative values of ADP and AMP were 3.2 and 3.3 fold that of ATP. The correlation between the adenylate assays and VBC is shown in Figures 2A, B. The samples containing large amounts of microorganisms tended to show high A3 values. If the benchmark is set at a typical value for high touch surfaces (e.g. 200 RLU), the ATP method showed many false-negatives, although these analytes were sampled before cleaning. These data indicate that the sensitivity of the ATP test may be insufficient for the hygiene monitoring of high touch surfaces in the hospital and the A3 test can better detect insufficient cleaning.

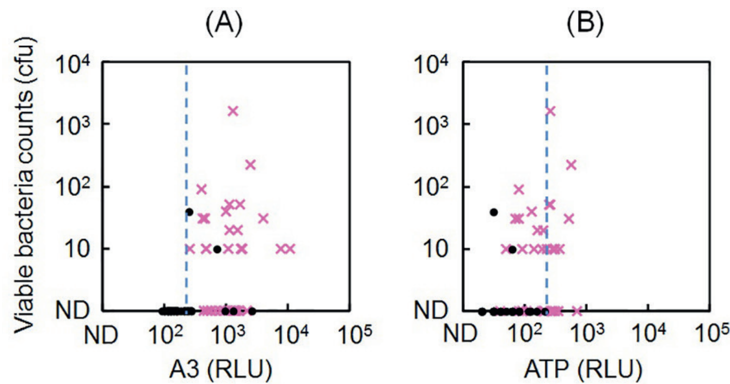
Tab. II. The inhibitory effects of disinfectant and cleaning agent to the A3 test.

| | Hydrogen peroxide (3%) | Enzyme-based immersion cleaning agent (1%) |
|---|---|---|
| ATP | 105 | 101 |
| ADP | 102 | 102 |
| AMP | 95 | 100 |

### Field study for the measurements of adenylates, protein and VBC on gastrointestinal reprocessed endoscopes

The RLUs of ATP, ATP + AMP, and A3 in debris from working channels of the gastroscopes (n = 19) and the colonoscopes (n = 6) immediately after endoscopic examination were measured and the ratios of the adenylate content of these samples are shown in Table I. Debris derived from endoscopes immediately after endoscopic examination generally contains digestive juices, mucous membranes, and sometimes blood. For the debris from the gastroscopes, the means of (ATP + AMP)/ATP and A3/ATP were 2.4 and 3.5, respectively. The relative values of ADP and AMP were 1.2 and 1.4 fold that of ATP. For the debris from the colonoscopes, the mean of (ATP + AMP)/ATP was 4.9 and A3/ATP was 7.6; the ratio of ATP:ADP:AMP was 1:2.7:3.9. These results indicated that the A3 test can detect inadequate cleaning with 3.5 and 7.6 times higher sensitivity in comparison with the conventional ATP test for gastroscopes and colonoscopes, respectively. The ratio of A3/ATP for colonoscopes was twice as large as that for gastroscopes, probably due to the difference in the expression levels of adenylate metabolizing enzymes, such as alkaline phosphatase in the intestine [16]. The correlations be-

Fig. 2. Correlations of viable bacterial count and the amount of adenylates on high touch surfaces (n = 35) before cleaning (pink cross) and after cleaning (black circle) with moistened microfiber cleaning cloth. RLU, relative light units. A) ATP + ADP + AMP (A3), B) ATP. The typical benchmark value showing a clean surface, 200 RLU, is shown with the light blue dotted lines.

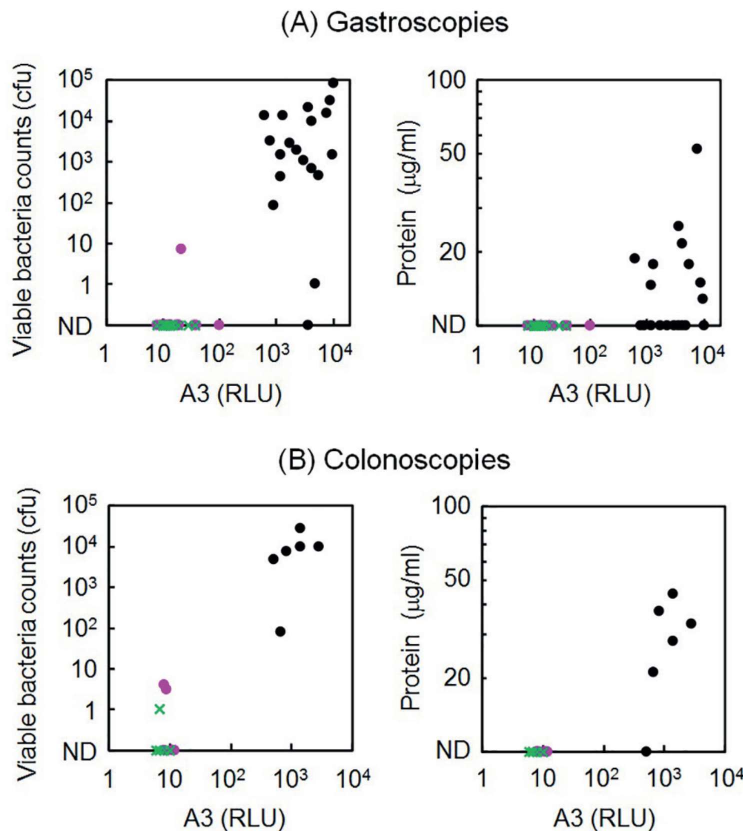



tween the protein assay, VBC, and the A3 test are shown in Figures 3A (gastroscopes), B (colonoscopes). As the cleaning steps progressed, the amount of A3 clearly decreased. Moreover, the samples that contained large amounts of protein and microorganisms tended to show high A3 values. The protein assay partly showed false-negative results although the analytes were sampled before cleaning. This data also suggests that the sensitivity of A3 method is higher than that of the Bradford protein assay.

## Discussion

ATP hygiene monitoring tests are widely used in many clinical and hospital applications for the prevention of HAI because they are easy to use and provide immediate results and verification of cleaning processes. However, this study demonstrated that testing ATP levels alone can be ineffective in verifying cleaning procedures for instruments with blood contamination and high touch surfaces because contamination may be overlooked. In

Fig. 3. Correlations of viable bacteria count, protein, and the amount of ATP + ADP + AMP (A3) in the working channels of the endoscopes after removal (black circle), manual cleaning (pink circle), and automated reprocessing (before alcohol flushing, green cross). RLU, relative light units. A) gastroscopes, B) colonoscopes. The protein concentration was measured by Bradford protein assay.



hemolysates, ATP can be promptly degraded to AMP through ADP, and AMP is accumulated (Fig. 1, Tab. I). In gloved-hand samples and on high touch surfaces, ATP only accounts for approximately 5% and 13% of A3, respectively. As a result, if the benchmark value is not stringent, the ATP method may produce false-negative results for cleaning of high touch surfaces, as shown in Figure 2B. On the other hand, the concentration of A3 remains relatively stable in hemolysates (Fig. 1), and the A3 test would provide higher sensitivity due to a positive signal even in the event of ATP degradation and is less likely to produce false-negative results for high touch surfaces (Fig. 2A). The detection sensitivities of the A3 method for residues derived from gastroscopes and colonoscopes were between 3 and 8 times higher than those of the ATP method (Tab. I). Moreover, the comparison with VBC and protein assays demonstrated that the A3 test can assess the reliability of cleaning procedures of endoscopes (Fig. 3). Thus, the A3 test provides a rapid, sensitive, and reliable method for monitoring environmental contamination and verifying cleaning procedures in hospital rooms, operating rooms, and in instrument reprocessing operations. Since adenylate concentrations are modulated in a complicated process by breakdown and synthesis in human body [17], the simultaneous detection of A3 as an indicator of contamination of body fluid is reasonable for cleaning verification in healthcare settings.

Disinfectants are used to kill microorganisms, and cleaning agents are used for the degradation of soil and for rust prevention in instruments. The A3 tests, performed after thorough washing and rinsing, and before disinfection/sterilization, can ensure proper washing to achieve effective disinfection/sterilization [4, 5]. However, since insufficient rinsing may leave chemical residuals on surfaces, data regarding inhibition (or lack thereof) of the A3 test by these chemicals is important. A previous study demonstrated that inhibition by major sanitizer compounds for the food industry, such as sodium hypochlorite (500 ppm of effective chlorine concentration), ethanol (ca. 80%), and benzalkonium chloride (0.1%) were not inhibitory when ca. 10% volumes of disinfectants were added [17]. Additional study also demonstrated that the presence of hydrogen peroxide (3%) and cleaning agents containing detergent, enzymes, e.g. protease, and rust preventive compounds had little effect on the A3 tests under the same conditions (Tab. II).

Field studies for high touch surfaces and endoscopes show that A3 values become smaller with progressive cleaning and microbial populations decrease (Figs. 2A, 3). Though the A3 swabbing test is not specific for the presence of bacteria (similar to conventional ATP tests), these data demonstrate that using A3 as an indicator for sufficient cleaning is a promising method for the prevention of HAI. This is simply because inadequate cleaning can give rise to the possibility that microorganisms remain on a surface or instrument. Furthermore, data show that A3 can be detected, despite VBC not being detected in both field studies (Figs. 2A, 3A). Three reasons can explain this result. One, the bacteria present on the surface could not survive the osmotic pressure of tap water in the swab or sampling solution (5% glucose) or were unculturable on TSA medium under the general conditions (aerobic, 35°C, 1 day). For example, blood agar plates may show growth of bacteria from clinical specimens that are unculturable on TSA [20]. Two, since small amounts of bacteria exist within (and are protected by) organic debris, swabbing the surface will not capture them. This is particularly important because organic debris can interfere with the activity of germicides for disinfection/sterilization and/or can protect microorganisms by acting as a physical barrier [4, 5]. Third, there were indeed no bacteria present but only organic matter remaining on the surface. Because it is known that hospital environments contain a diverse range of bioaerosols, which include bacteria and fungi, it would be expected that these bioaerosols contribute viable cells, including opportunistic pathogens, that can adhere to, be protected by, proliferate, and form biofilms within any residual organic debris remaining [21]. Taking into consideration the cases mentioned above, the high A3 values, even in the absence of small amounts of bacteria, should be seen not as false-positive, but indicative of inadequate cleaning and as a risk for HAI.

## Conclusions

Because the A3 test can detect total adenylate simultaneously, it provides a more sensitive and reliable indicator of cleanliness in hospital rooms, operating rooms, and in instrument reprocessing operations than the conventional ATP test. Since the A3 test is easy to use and provides immediate results and verification of cleaning processes, it guides and assures better sanitation outcomes and supports a more effective hygiene program in healthcare settings.

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## Conflict of interest statement

Mikio Bakke is an employee of Kikkoman Biochemifa Company. Shigeya Suzuki is an employee of Kikkoman Corporation. E. Kirihara and S. Mikami declare that there is no conflict of interest regarding the publication of this paper.

## Authors' contributions

MB conceived, designed the research, collected data, wrote the manuscript, SS planned the method, EK and SM coordinated the field studies in the hospital. All au-

thors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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