〈Report〉

Adenosine triphosphate as a useful measure of the hygiene status in a pathological laboratory

Yosuke Tajika[1,2,*], Yuki Nakajima[1], Megumi Orita[1], Kouji Kobayashi[3], Masayo Tida[2] and Hideaki Suzuki[4]

Summary This study aimed to determine the hygiene status of a pathology laboratory. We measured the level of adenosine triphosphate (ATP), a potential indicator of microbial contamination, before and after performing laboratory work. Specific points on the cutting table and the organ imaging table were cleaned with 70% ethanol and paper towels for a fixed period at the beginning and end of the routine. Hand disinfection was performed with triclosan- or isopropylmethylphenol-containing soap. ATP activity was measured before and after cleaning. Cleaning reduced the ATP activity level on both tables, and the ATP activity level decreased on the cutting table as the number of cleanings increased. Triclosan-containing soap was associated with a greater decrease in ATP activity after handwashing. Workstation cleaning and handwashing protocols reduced the level of ATP activity in a pathology laboratory. Our results suggest that ATP can be used both as a cleanliness index and as a measure for monitoring contamination.

Key words: Pathological examination, Infection control, Adenosine triphosphate (ATP) activity, Occupational health

1. Introduction

Pathologic examination involves the direct handling of organs and biopsy specimens and thus carries a high risk of infection. However, few reports have addressed infections related to pathology laboratories.[1] According to Masuda et al., the average annual infection rate associated with clinical laboratory work was 0.2% over a 10-year period. In that study, Mycobacterium tuberculosis was the most common infectious agent, followed by hepatitis B virus.[2] Consistent with that observation, laboratory-acquired infections, such as tuberculosis and

[1]Department of Pathology, Saiseikai Toyama Hospital, 33-1 Kusunoki, Toyama-shi, Toyama, 931-8533, Japan.
[2]Infection Control Team, Saiseikai Toyama Hospital, 33-1 Kusunoki, Toyama-shi, Toyama, 931-8533, Japan.
[3]Department of Medical Technology, Kitasato Junior College of Health and Hygienic Sciences, 500 Kurotsuchi Shinden, Minami Uonuma, Niigata, Japan.
[4]Department of Clinical Laboratory Sciences, School of Health Sciences, Fukushima Medical University, 1 Hikarigaoka, Fukushima-shi, Fukushima, Japan.

[*]Corresponding author: Yosuke Tajika, Saiseikai Toyama Hospital, 33-1 Kusunoki, Toyama-shi, Toyama, 931-8533, Japan.
Tel: +81- 76-437-1111
E-mail: y.tajika@saiseikai-toyama.jp

Received for publication: October 4, 2021
Accepted for publication: January 13, 2022

hepatitis B infection, occur frequently during laboratory work, and pathologic specimens have been identified as the causes of some infections. The occurrence of laboratory-acquired infections underscores the difficulty of preventing infection and the need for infection control according to the risk level.[3]

The Centers for Disease Control and Prevention manual recommends that a pathology laboratory should implement biosafety level 2 measures based on the assumption that pathologic specimens contain pathogenic microorganisms.[4] In particular, such measures should be applied to the cutting table in the pathology examination room, and care should be taken to clean this table after tissue processing has been completed. In this study, we used adenosine triphosphate (ATP) activity as an index of the degree of microbial contamination in a pathology laboratory before and after tissue processing to determine whether ATP activity is a useful measure of the degree of hygiene after cleaning the cutting table and handwashing.

2. Materials and Methods

Two areas, the cutting table and the imaging table, were evaluated in this investigation of the hygiene status of a pathology worksite. ATP activity on these surfaces was measured once, before and after cleaning the surfaces at 2, 3, 7, and 8 h from the beginning of the routine. The experiments were conducted on the day before without washing. The ATP activity measurement points were determined before and after cleaning the surfaces. At the end of the routine, cleaning was repeated, and ATP activity was measured again. During work, the table was cleaned by spraying the whole surface with 70% ethanol and then wiping the surface with paper towels. At the end of the routine work, 10 cm^2 section of each work area was sprayed with 70% ethanol, and then the same area was swabbed with cotton swab twice with the same pressure horizontally and vertically to collect samples for measuring ATP activity. The effectiveness of the usual handwashing method was determined by evaluating the hands of three volunteer pathology examiners who had provided informed consent. ATP activity on the hands was measured before and after washing with two types of hand soap: soap containing triclosan (Clean Chemical Co., Ltd., Osaka, Japan) and soap containing isopropylmethylphenol (Albose Co., Ltd., Osaka, Japan). The evaluation was performed twice for each examiner on different study days. After washing the hands and drying them with paper towels, the palm of the dominant hand from the wrist to the fingertips and from the thumb to the little finger and the spaces between the fingers were wiped five times with a swab.

The sanitary environment was evaluated by measuring the ATP produced by bacteria. A Clean Trace ATP activity kit (3M Healthcare Co., Ltd., MN, USA) was used for this purpose. The coefficient of variation for reproducibility of this kit as indicated by the manufacturer was 7.4 %, and the limit of detection was 30 Relative Light Unit (RLU). The wiped swab was inserted into a tube containing the test reagent and stirred to initiate a reaction. Next, the swab was mounted in a UNG3 luminometer (3M Healthcare Co., Ltd.), and ATP activity was measured to quantify the degree of contamination.

All data are presented as ATP activity values per cm^2 of wiped area, and the means ± standard deviation (SD). The statistical significance of differences was evaluated by Wilcoxon matched-pairs signed rank test using GraphPad Prism version 9.3.1. *P*-values less than 0.05 were considered statistically significant.

3. Results

First, the ATP activity levels on the cutting table and organ imaging table used for pathologic examinations were measured to evaluate their hygiene status. The ATP activity levels on the cutting table before and after performing work were 134 and 189 RLU /cm^2, respectively, at 2 h, 38 and 33 RLU/cm^2 at 3 h, 46 and 61 RLU/cm^2 at 7 h, and 48 and 20 RLU/cm^2 at 8 h. A value of 18 RLU/cm^2 was measured at the final cleaning.

The ATP activity levels on the organ imaging

table before and after performing work were 786 and 686 RLU/cm^2, respectively, at 2 h, 483 and 223 RLU/cm^2 at 3 h, 303 and 254 RLU/cm^2 at 7 h, and 294 and 317 RLU/cm^2 at 8 h. A value of 42 RLU/cm^2 was measured at the final cleaning (Fig. 1).

Next, the ATP activity levels were measured after handwashing with different cleaning agents. The average values measured before and after washing with soap containing isopropylmethylphenol were 1,522 and 319 RLU/cm^2, respectively, indicating a considerable decrease in ATP activity. The average values measured before and after washing with soap containing triclosan were 2,004 and 310 RLU/cm^2, respectively. Although neither of these differences was significant, all the results suggested that ATP activity significantly decrease after handwashing ($P < 0.05$; Fig. 2). For the purpose of hand hygiene, the ATP activity level before washing was set at 100% with soap containing triclosan or isopropylmethylphenol, and the rate of reduction of ATP activity after washing was calculated. The ATP activity level decreased by an average of 11.2% after washing with soap containing triclosan and 18.1% after washing with soap containing isopropylmethylphenol.

4. Discussion

In this study, we evaluated ATP activity as a potential measure of contamination by living organisms on the workbenches and the hands of examiners in a pathology laboratory. Hygienic and environmental monitoring is important for preventing cell contamination and maintaining infection control during pathologic work.[5] Generally, ATP activity is used as an objective measure of hygiene status in the processing of foods and drinks such as melon, raw milk, and drinking water.[6-8] In contrast to the long period required for culture of a general wipe test, ATP activity can be measured within 30 min and is highly sensitive.[9] Because ATP is present in the human body, we thought that it could be evaluated as an indicator not only of bacterial contamination but also of cell contamination. In contamination by human cells, it is difficult to distinguish between the patient's cells and those from someone other than the patient. For example, contamination of the thyroid gland by squamous epithelial cells results in an erroneous determination. In our experiments, we found that wiping with 70% alcohol reduced the

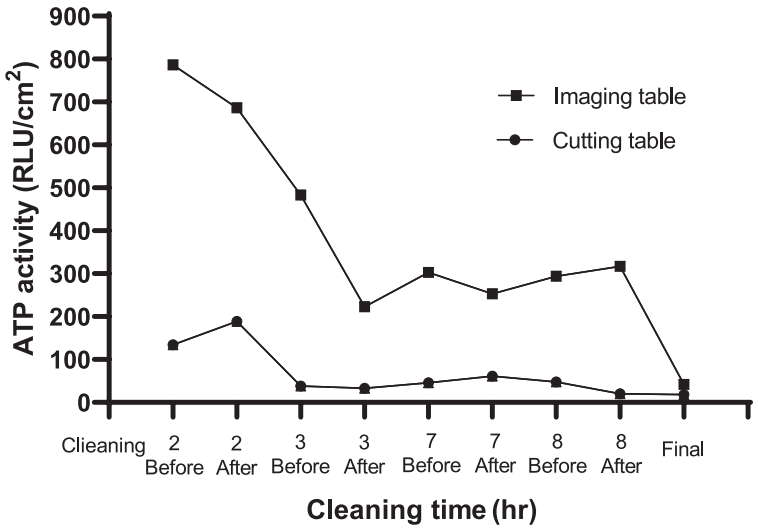

Fig. 1. Changes in adenosine triphosphate (ATP) activity level with cleaning protocols over time in a pathology laboratory.
The ATP levels of the cutting (●) and imaging (■) table samples were measured at the beginning and end cleaning. A portion (10 cm^2) of each table were wiped with cotton swab and measured only once using Clean Trace ATP activity kit.

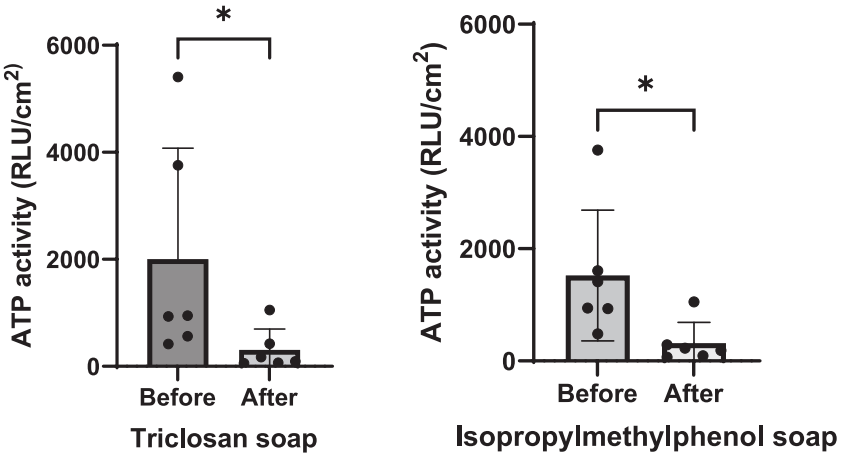

Fig. 2. Changes in adenosine triphosphate (ATP) activity level on the hands of pathologic examination workers before and after handwashing, according to the antibacterial agent contained in the soap.
The effects of handwashing with soaps containing triclosan (a) and isopropylmethylphenol (b) were evaluated. The hands of three volunteers were measured twice each on different days and measured only once using Clean Trace ATP activity kit (n=6). *P < 0.05

ATP activity level on the laboratory workbenches and handwashing with triclosan-containing soap was effective in terms of good hand hygiene. Because ATP is present in human-derived cells, we believe that the ATP activity level can be used to monitor contamination by other cells, which could affect the accuracy of a pathologic examination. Our results suggest that the ATP activity level is a useful measure of laboratory hygiene.

We selected the cutting table and the organ imaging table as monitoring locations because both often come into contact with fresh materials during and after pathologic evaluations, which may cause contamination and infection. We observed to decrease ATP activity on the imaging table after cleaning; the decrease in ATP activity on the cutting table was not very pronounced, but the ATP activity level became lower with each increase in the number of washes. Beatrice et al. defined 100 Colony forming unit (CFU)/ cm^2 or 40 RLU/cm^2 as the threshold for the medium-risk category and 250 CFU/cm^2 or 50 RLU/cm^2 as the threshold for the low-risk category.[10] Our results showed that the number of washes was associated with the low-risk category. For the cutting table, the low-risk category was reached after three washes, and for the imaging table, the low-risk category was reached after nine washes. This time, wiping was done every 8 times in 8 hours. Even after wiping, the work continued, and therefore, it became contaminated. Therefore, we think that regular wiping is necessary. In our study, even the highest ATP activity values measured on the cutting table, which had a stainless-steel surface, were lower than those reported in the literature related to food processing, and were considerably lower than previously reported values in the case of washing followed by wiping.[11] Our results may have been due to the use of continuous ventilation, which prevented an increase in the ambient temperature, and performance of cleaning throughout the day. Accordingly, the initial degree of microbial contamination was low, and the related ATP activity level remained low. However, ATP activity on the cutting table was highest before the beginning of the routine daily work and decreased thereafter. It was also higher than the reported food bench ATP activity except before the afternoon works and during the final wipe.

In September 2016, the US Food and Drug Administration announced that it would ban products containing 19 antimicrobial agents, including triclosan and triclocarban.[12] However, triclosan remains an ingredient in hand sanitizers used in

medicine and is considered beneficial for improving compliance with handwashing and hand sanitizer use.[13] The effects of triclosan on the skin are both sustained and cumulative, which may reduce ATP activity on the hands.[12] Isopropylmethylphenol is effective against the bacterial species *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Vibrio cholerae*, and *Streptococcus pyogenes*, as well as the fungal species *Candida albicans*.[14] This chemical is also used as an antiperspirant. Despite these observations, there is little evidence to support the benefits of soap containing antimicrobials compared to regular soap, and long-term exposure appears to affect bacterial resistance and as endocrine disruptors.[12] In this study, we evaluated the disinfection effect of triclosan and isopropyl methylphenol. The results showed that both triclosan and isopropylmethylphenol decreased ATP activity and were considered to have disinfectant effects.

Our study had some limitations. For example, the sources of laboratory-related infection remain unclear. However, we believe that evaluation of the hygiene status of the laboratory table used to handle raw samples during the first cutting procedures is a useful preventive measure. In this study, hygiene status was evaluated according to ATP activity. However, these measures were not compared with data from standard microbial culture examination. In future evaluations, a comparison of these types of data would yield more accurate results. Furthermore, we observed variations in ATP activity, even on the hands of the same subjects, before handwashing with different disinfectants. More concrete results could be obtained from further comparative studies with adjustment for variations. In the future, we aim to perform these examinations and to provide reliable experimental data.

Conflicts of interest

The authors have no conflicts of interest.

Acknowledgments

We wish to thank members of department of pathology, Saiseikai Toyama Hospital for advice on experimental design.

References

1. Shimazu K, Fujinaka H, Matsui M, Sasaki S, Shimoyama R, Nishikawa K and Fushimi H: Health and safety considerations in our pathology laboratory renovation: focusing both formaldehyde and airborne infection control measures [Jpn]. Med J Osaka Gen Med Cen (Osakafuritukyuuseiki Sougouiryou center Igakushi), 35: 33-38, 2012.
2. Masuda T and Isokawa T: Biohazard in clinical laboratories in Japan. J Jpn Assoc Infectious Dis, 65: 209-215, 1991.
3. Huasong P, Muhammad B and Hafiz MN Iqba: Improved biosafety and biosecurity measures and/or strategies to tackle laboratory-Acquired infections and related risks. Public Health, 15: 2697, 2018.
4. Edited by Paul JM and Jeffrey P: CDC biosafety in microbiological and biomedical laboratories 6th. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health, USA, 5: 33-38, (2020)
5. Cooper RA, Griffith CJ, Malik RE, Obee P and Looker N: Monitoring the effectiveness of cleaning in four British hospitals. Am J Infect Control, 35: 338-341, 2007.
6. Ukuku D, Sapers G and Fett W: ATP Bioluminescence assay for estimation of microbial populations of fresh-cut melon. J Food Protection, 68: 2427-2432, 2005.
7. Samkutty PJ, Gough RH, Adkinson RH and McGrew P: Rapid assessment of the bacteriological quality of raw milk using ATP bioluminescence. J Food Protection, 64: 208-212, 2001.
8. Boe-Hansen R, Martiny AC, Arvin E and Albrechtsen HJ: Monitoring biofilm formation and activity in drinking water distribution networks under oligotrophic conditions. Water Sci Technol, 47: 91-97, 2003.
9. Beckers B and Lang HR: Rapid Detection of bacteriuria in bacteriological routine laboratory. Zentralbl Bakteriol Mikrobiol Hyg A, 254: 515-520, 1983.
10. Beatrice C, Benedetta T, Michele T, Francesco A, Angelo B and Gaetano P: Evaluation of the cleaning

procedure efficacy in prevention of nosocomial infections in healthcare facilities using cultural method associated with high sensitivity luminometer for ATP detection. pathogens, 7(3): 71, 2018.

11. G Tebbutt, V Bell, J Aislabie: Verification of cleaning efficiency and its possible role in programmed hygiene inspections of food businesses undertaken by local authority officers. J App Mic, 102(4): 883-1176, 2007.
12. Edited by FDA: FDA issues final rule on safety and effectiveness of antibacterial soaps. U.S. Food and Drug, USA, https://www.fda.gov/news-events/press-announcements/fda-issues-final-rule-safety-and-effectiveness-antibacterial-soaps (accessed 2021.9.30)
13. Weatherly LM and Gosse JA: Triclosan exposure, transformation, and human health effects. J Toxicol Environ Health B Crit Rev, 20: 447-469, 2017.
14. Ezoubeiri A, Gadhi CA, Fdil N,et al.: Evaluation of the cleaning procedure efficacy in prevention of nosocomial infections in healthcare facilities using cultural method associated with high sensitivity luminometer for ATP detection.. J Ethnopharmacol, 99: 287-292, 2005.