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Establishment of a rapid and quantitative method for detecting the range of infection exposure in preclinical dental education

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Abstract

Backgrounds Safe dental treatments that prevent nosocomial and cross-infections are essential for patients and dental workers. However, dental students sometimes pay inadequate attention to infection control, especially in preclinical practice, because of too much focus on technical training, such as the use of equipment, etc. The spread of infections such as SARS-CoV-2, antibiotic-resistant bacteria, and oral bacteria are sometimes lethal for medically compromised patients. Thus, the rapid and inexpensive detection system to detect and measure dental practice-related infection spread during preclinical treatment is highly desired for dental education. This study aimed to establish a method to quantify and visualize infected areas using dental phantoms for safe and effective preclinical dental practices.

Methods At first, we developed artificial saliva as an in vitro study, including food-derived bacteria and fluorescence dye, which is safe for application to preclinical practice education. In vitro study, the correlation between adenosine triphosphate (ATP) levels and *Lactobacillus* colony numbers in yogurt was examined using the ATP fluorescent method, with colony counting on yogurt only and a mixture of yogurt and ultraviolet (UV)-sensitive hand lotion. The mixed liquid of yogurt and hand lotion was used as artificial saliva. Second, we used this artificial saliva in preclinical education. The degree of contamination of personal protective equipment and dental chairs in preclinical practice using this artificial saliva was determined using the ATP fluorescent method and measuring the luminescence areas among 10 dentists, 10 dental residents, and 10 fifth-grade dental students.

Results ATP levels and *Lactobacillus* colony numbers in yogurt were positively correlated with yogurt alone and a mixture of yogurt and UV-sensitive hand lotions (correlation coefficient ≈ 1). Preclinical education using a mixture of artificial saliva successfully quantified and visualized infectious areas and droplets, which revealed significant differences in ATP amounts in personal protective equipment among groups according to years of experience as dental practitioners ($p < 0.05$).

Conclusions An education system for infection control constructed using artificial saliva containing *Lactobacillus* and a UV-sensitive fluorescent hand lotion quantified the infectious areas and degrees. Thus, this method is effective in preclinical practice using dental phantoms.

Keywords Dental education, Infection control, Fluorescent dye, Adenosine triphosphate, *Lactobacillus*

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Background

Infection prevention measures in dental practice are based on standard precautions according to the guidelines issued by the Centers for Disease Control and Prevention [1]. It has become increasingly crucial that dental workers ensure safe dental treatment to prevent nosocomial and cross-infections due to the spread of emerging infectious diseases, such as coronavirus disease 2019 (COVID-19) [2–8]. Despite this reality, some reports suggest that dental students' knowledge and practices regarding measures against infection control are inadequate [9–11]. Thus, the preclinical curriculum related to infection control measures for dental students should be enhanced [12]. However, the reality is that pre-clinical education for infection control in Japan is generally lecture-based and does not involve practical training.

When handpieces and ultrasonic scalers are used in dental practice, aerosols sometimes cause aerial infections and infectious droplets around dental chairs [13, 14]. The droplet ranges and degrees vary according to the practitioner's skill level, highlighting the need for personal protective equipment (PPE) [15–17]. Other reports suggest dental equipment may be indirectly exposed to infected PPE including gloves [18, 19]. Visualizing and quantifying the degree of infection would contribute to the improvement in awareness of dental workers and students in managing infection; however, such an attempt at preclinical dental practice using phantoms has not yet been reported.

The adenosine triphosphate (ATP) fluorescence assay has been widely used to examine contamination in hospitals and the hygienic conditions of food [20–22]. Ultra-violet (UV)-sensitive fluorescent hand lotions have been developed to check for inadequate hand hygiene educationally [23]. A report shows that educational intervention using a graphical assessment method with fluorescent hand lotions improved students' hygiene practice [24]. In this way, a teaching aid for pre-clinical dental education for infection control was devised, combining the ATP fluorescence assay method for quantification with hand lotion that reacts with UV light for visualization. *Lactobacillus* species were selected in the ATP fluorescent assay because they are readily available as commercial yogurt as daily food. The research question was whether we could utilize inexpensive, rapid, and practical evaluation methods for educating dental workers, including students. Thus, we explored the efficacy of a combination of yogurt and UV-sensitive fluorescent hand lotions as artificial saliva and verified the validity of preclinical education in infection control using artificial liquid saliva.

Materials and methods

In vitro study

Lactobacillus ATP in yogurt

The ATP amounts in yogurt on plastic surfaces were measured in vitro and the possibility of applying bacterial ATP in yogurt to an infection control system was assessed. ATP amounts in the yogurts commercially available Meiji Bulgaria Yogurt® (Meiji, Co., Ltd., Tokyo, Japan) and Makibano Asa Yogurt® (Megmilk Snow Brand Co., Ltd., Tokyo, Japan) were measured using Lumitester Smart® and LuciPac A3 Surface® (Kikkoman Biochemifa Company, Tokyo, Japan) [25]. The yogurts were stirred for 30 s and diluted serially up to 0.001% with phosphate buffer solution (PBS; Thermo Fisher Scientific, MA, USA) to adjust to find the appropriate concentration and viscosity. 100 µL of diluted yogurt solution was dropped on two fingers and applied on 5×5 cm square plastic sheets by those fingers for 5 s. Yogurt samples applied on plastic sheets were rubbed with applicators of LuciPac A3 Surface® after dipping the applicators into PBS for 5 s. ATP amounts in collected samples were measured using Lumitester Smart®, according to the manufacturer's brochure.

Colony numbers of *Lactobacillus* species in yogurt

Viable bacterial colonies of the yogurt samples in Sect. 2.1.1 were counted on agar plates to confirm the presence of viable *Lactobacillus* species. A solution of 100 µL of diluted yogurt with PBS up to 0.00001% was seeded on the MRS agar plates (Solabia Biokar Diagnostics, Pantin, France). The plates were anaerobically incubated at 37°C for 72 h, and colonies were counted.

A mixture of hand washing educational lotion and yogurt

To investigate the possibility of using a mixture of UV-sensitive fluorescent hand lotions and yogurt for artificial saliva, bacterial ATP levels in the mixture were measured. Specifically, Hand Washing Checker Lotion® (Saraya, Osaka, Japan) was mixed in Meiji Bulgaria Yogurt®, one of the yogurts used in Sect. 2.1.1, diluted serially up to 0.001% with PBS and stirred for 30 s. The 100 µL mixture of yogurt and hand lotion was dropped on two fingers and applied on 5×5 cm size of square plastic sheets by those fingers for 5 s. Mixture samples applied on plastic sheets were rubbed with applicators of LuciPac A3 Surface® after dipping the applicators into PBS for 5 s. Bacterial ATP amounts in those samples were detected using Lumitester Smart® in accordance with the manufacturer's protocol.

Trial for clinical education

Research subjects

This study was approved by The Okayama University Ethics Committee (Approval No. 2308-032). We recruited 10 participants each from dentists, dental residents, and preclinical fifth-grade dental students from the Okayama University Hospital (30 participants). All the participants provided written informed consent. Individuals allergic to yogurt or latex were excluded. The background of the participants, including sex, age, and clinical experience, are presented in Supplemental Table 1.

Preclinical practice using dental phantom

Periodontal disease jaw models in which artificial plaque (Nisshin Dental Products Inc., Kyoto, Japan) was applied to the tooth surface, and the buccal mucosa model (Nisshin Dental Products Inc.) was equipped with a dental phantom (Morita Corp., Osaka, Japan). A set of dental mirror, forceps, explorer, and 40 cm lengths of dental floss was prepared on the tray of the dental bracket table. Four-mL mixture of yogurt and Hand Washing Checker Lotion (mixed ratio was 1:3) as artificial saliva previously described in Sect. 2.1.3 was dropped on the tongue and applied all over the oral cavity of the dental phantoms immediately before the start of this preclinical practice (Fig. 1A). A document intended for oral assessment was placed on a 1-m width desk. During the assessment, the participants were required to wear surgical masks (175×90 mm; Hasegawa Menko Co., Ltd., Nagoya, Japan), face shields (Yamazaki Corp., Osaka, Japan), medical caps (Hogy Medical Co., Ltd., Tokyo, Japan), plastic aprons (Nishikin Co., Ltd., Tokyo, Japan), and gloves (O & M Halyard Japan, G.K., Tokyo, Japan), as shown in Fig. 1B (representation of the protective gear). Subsequently, they conducted an oral assessment for 5 min, periodontal examinations (periodontal pocket probing, tooth mobility assessment [26], and plaque control records [27]) for 15 min, and flossing for 5 min. Three evaluators monitored the participants during the practice.

Evaluation of outcomes

The primary outcome was the amount of bacterial ATP that adhered to surgical masks and face shields. The fluorescent areas appraised secondary outcomes and contact frequency of participants on surgical masks and face shields, as well as the presence of fluorescent areas on the desk, operators' chairs, plastic aprons, medical caps, and pencils.

During the training session with the phantom, all surgical masks and face shields were collected and placed in a box with dimensions of 24 cm × 39 cm × 32 cm. The box had a UV light (Hand Washing Checker Light emitting diode (LED); Saraya) installed on the top or bottom. The surgical masks and face shields were then photographed.

To evaluate the primary outcome, avoiding any varied handling, only one specific of the three evaluators swabbed the surfaces of the surgical masks and face shields using applicators of LuciPac A3 Surface® after dipping them into PBS for 5 s. The bacterial ATP amounts were detected using Lumitester Smart®. Three evaluators confirmed the presence of a fluorescent area on the desk, operator chairs, plastic aprons, medical caps, and pencils. In addition, all three evaluators observed the contact frequency of each subject. The fluorescent areas on the captured photos of the surgical masks and face shields were assessed with the average by two blinded evaluators of the three using the software ImageJ® (National Institutes of Health, Bethesda, Maryland, USA).

Statistical analysis

The appropriate comparisons were made using the T-test, ANOVA, Fisher's exact test, Kruskal-Wallis test, and Wilcoxon test. Statistical analysis was performed using JMP® software (JMP Statistical Discovery LLC., NC, USA). Statistical significance was set at $P < 0.05$.

Results

Lactobacillus ATP amounts and colony numbers in yogurt were correlated

The amount of bacterial ATP in both yogurt samples varied with a concentration gradient. The detection limit was 0.001% (Fig. 2A). Colonies in 1–0.00001% of yogurts were countable and gradually decreased along the concentration gradient. No significant differences were observed in the number of colonies between the two yogurt types (Fig. 2B). The bacterial colony numbers and ATP levels were positively correlated (Fig. 3A, B). Meiji Bulgaria Yogurt was used for further study based on the results and availability.

Lactobacillus ATP in yogurt mixed with fluorescent hand lotion were detectable

The amount of ATP in diluted yogurt mixed with 50% hand lotion was detectable, similar to yogurt alone (Fig. 4A). However, the luminescence of 50% hand lotion was invisible and could not be confirmed using an optical camera (data not shown). Therefore, the bacterial ATP levels in a mixture of 75% UV-sensitive fluorescent hand lotion and 25% yogurt were measured (Fig. 4B), which clarified concentration-dependent measurement values.

Lactobacillus ATP on mask and face shield decreased depending on clinical experience

The median *Lactobacillus* ATP levels on surgical masks decreased with years of clinical experience. Specifically, the median was 443.0 relative light units (RLU) overall, with values of 583.5 RLU for dentists and 784.5 RLU for

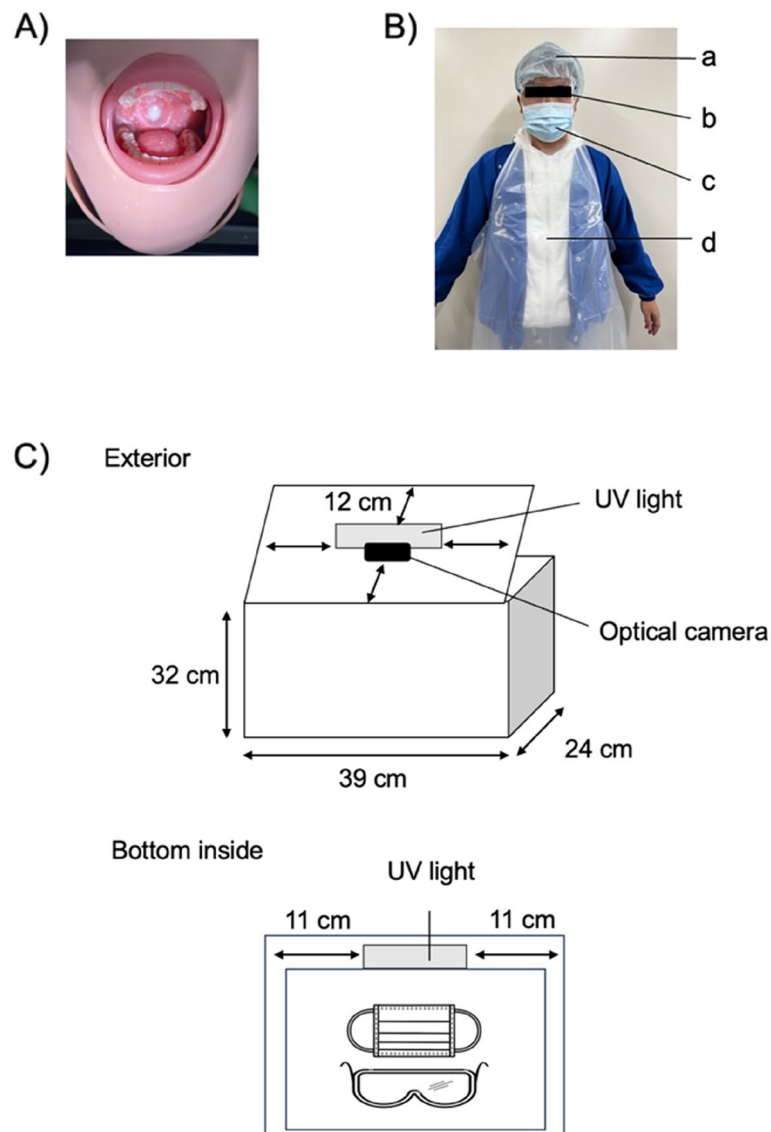


Fig. 1 Preparation for preclinical practice and device for measurement. **A** Dental phantom: A mixture of yogurt and a UV-sensitive fluorescent hand lotion is applied to the oral cavity of the dental phantom. **B** Preparation of PPE: The participants wear a) a medical cap, b) face shield, c) surgical mask, and d) apron before starting this practice. **C** Photograph device with UV-light: The photographic device is originally constructed from a box with a length of 39 cm, width of 24 cm, and vertical length of 32 cm. UV light is placed on the upper side of the masks and the bottom of the face shields, and photos are captured from the window on the upper side

dental residents (Fig. 5A); however, these differences were not statistically significant. In contrast, the median *Lactobacillus* ATP levels on face shields also decreased with clinical experience and showed statistical significance. The median values were 101.0 RLU for dentists, 172.0 RLU for dental residents, and 588.0 RLU for dental students (Fig. 5B).

The luminescence area on the mask and face shield were similar among the three groups

No participant touched their masks or face shields directly. Nevertheless, fluorescent droplets mainly originated from flossing attached to PPE. Those fluorescent droplet areas measured using ImageJ[®] had no significant differences among all groups although the areas of dental students tended to be larger than those of the other two

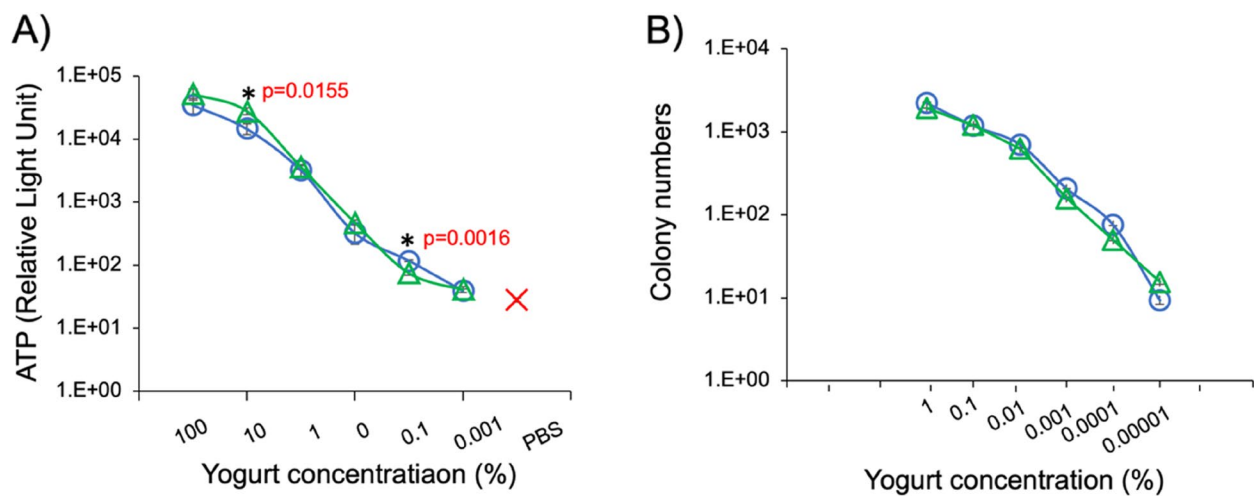


Fig. 2 *Lactobacillus* ATP amounts and colony numbers in yogurt. **A** ATP amounts in yogurt: ATP amounts in 100 μ L of yogurts are measured. The data are presented as the mean $\pm$ standard deviation (SD) from three independent experiments. O: Meiji Bulgaria Yogurt, Δ : Makibano Asa Yogurt, $\times$: PBS (solvent; background). **B** *Lactobacillus* colony numbers in yogurt: Colonies in 100 μ L of yogurts are counted on agar plates. The data are presented as the mean $\pm$ SD from three independent experiments. O: Meiji Bulgaria Yogurt, Δ : Makibano Asa Yogurt. Student's t-test was performed between two yogurts at the same concentration. Significant differences were found between them at 10% and 0.1% concentrations (**A**)

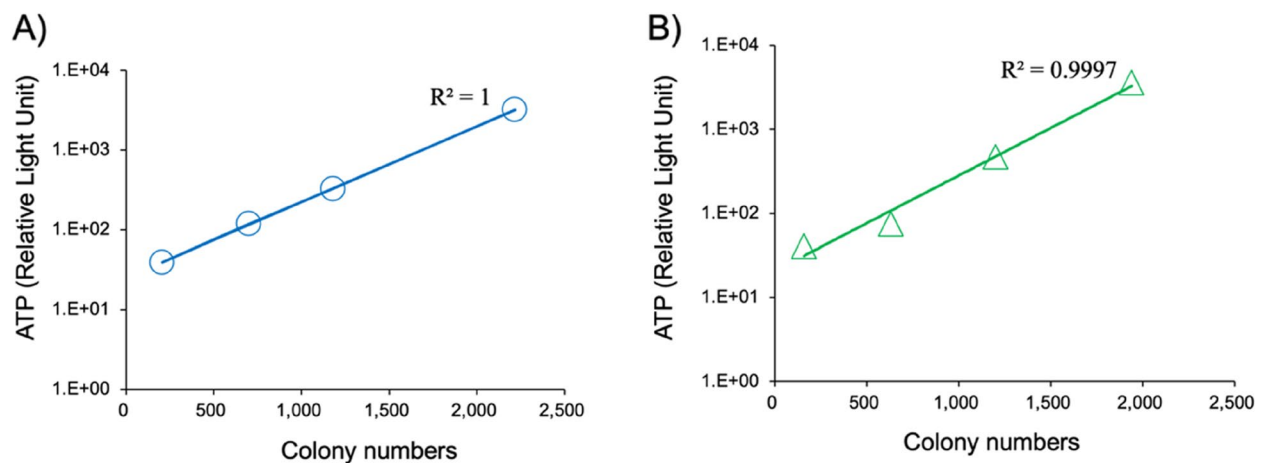


Fig. 3 Correlation between *Lactobacillus* ATP amounts and colony numbers in yogurt. The Pearson's correlation coefficients between bacterial ATP levels and colony number were analyzed. Coefficient of determination (R^2) was shown. Both yogurt types showed a positive correlation. **A** Correlation in yogurt, Meiji Bulgaria Yogurt; $P = 0.0812$. **B** Correlation in yogurt, Makibano Asa Yogurt; $P = 0.1155$

groups (Fig. 6). The number of participants whose surroundings contained fluorescent droplets or contacts is shown in Table 1. A significant difference was observed only in the operators' chairs.

Discussions

There have been various reports on cross-infection and aerosol contamination of PPE during dental treatment, and it is crucial to prevent cross-infection and to use PPE correctly in hospitals. Viable bacterial counts on agar media [28] and mass spectrometry [29, 30] were used to

collect PPE during dental treatment to investigate and detect contamination around dental chairs and PPE. However, the problem is that detecting the bacteria takes a long time. In addition, a laser spectrometer was used to examine the amount of aerosol during dental treatment using a phantom, but it could not be confirmed by the naked eye [31]. On the other hand, the compliance of dental healthcare professionals to the correct placement of PPE is still insufficient, and it is necessary to acquire correct knowledge from the educational process [15]. In other words, developing a method to teach the

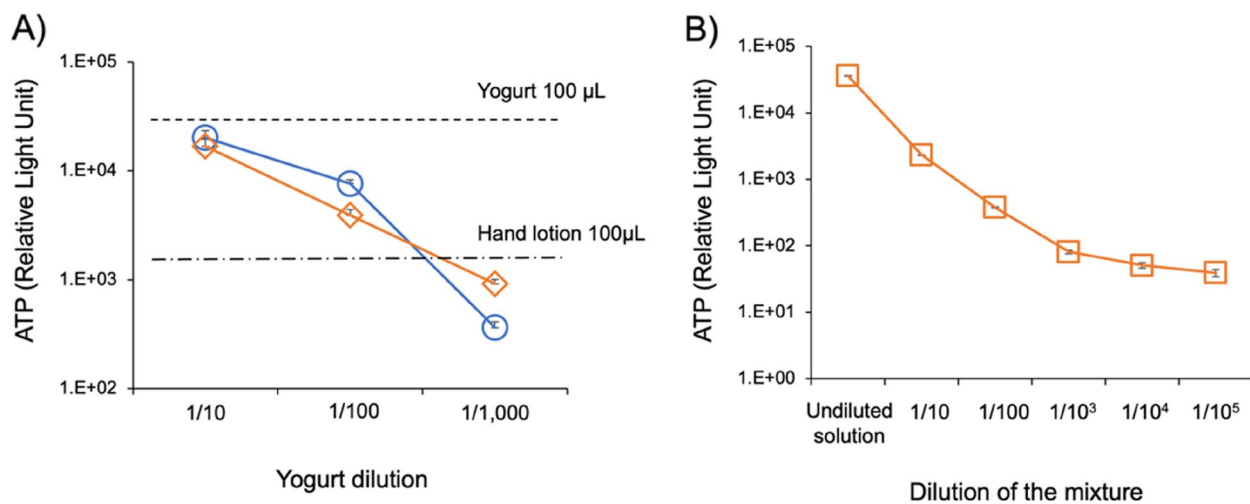


Fig. 4 *Lactobacillus* ATP amounts in a mixture of yogurt and fluorescent hand lotion ATP levels are measured in a mixture of yogurt and UV-sensitive fluorescent hand lotion. The data are presented as the mean $\pm$ SD from three independent experiments. **A** Mixture with the ratio 1:1 $\diamond$: mixture, $\circ$: yogurt only The dashed line means ATP amounts in 100 μ L of undiluted yogurt or hand lotion. **B** Mixture with the ratio 1:3 (yogurt: hand lotion)

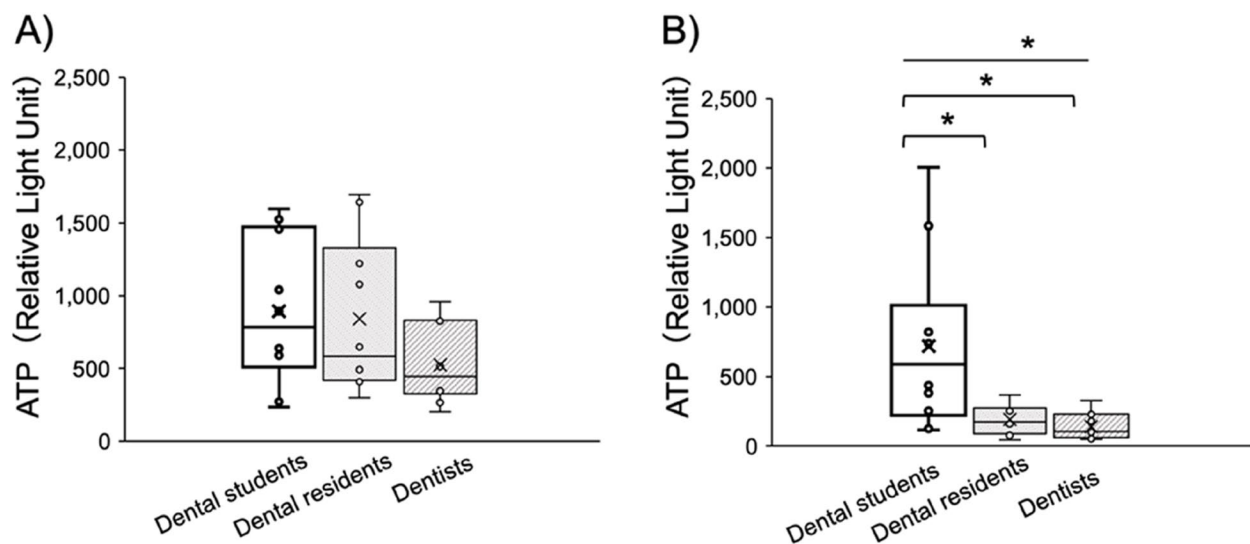


Fig. 5 *Lactobacillus* ATP amounts on PPE. The amount of *Lactobacillus* ATP in PPE in a trial for clinical education is shown. Data from the three groups (10 participants each from dentists, dental residents, and dental students) are analyzed using the Kruskal-Wallis test, and between the two groups using the non-parametric Wilcoxon test. *: $p < 0.05$. **A** ATP amounts on the surface of the surgical mask: There is no significant difference among groups. **B** ATP amounts on the surface of the face shield: There is a significant difference among the three groups ($p=0.0033$). The comparisons between dental residents and students ($p=0.0140$) and between dentists and students ($p=0.0022$) show significant differences

importance of PPE safely, quickly, and practically during phantom practice in educational courses is necessary.

This study aimed to investigate the potential of a mixture of yogurt and UV-sensitive fluorescent hand lotions as artificial saliva using ATP measurements and the presence of fluorescent areas. Secondly, preclinical dental training using the devised artificial saliva could be useful in detecting infectious areas.

In vitro study showed that *Lactobacillus* ATP levels in yogurt were positively correlated with colony numbers. Although coefficients of determination were so close to 1, P values were less than 0.05, meaning insignificant. Because the number of samples is small (4 each), there was no “statistical significance”, but there was a possibility of “practical significance”. Furthermore, ATP levels were able to be measured even in a mixture of yogurt and hand

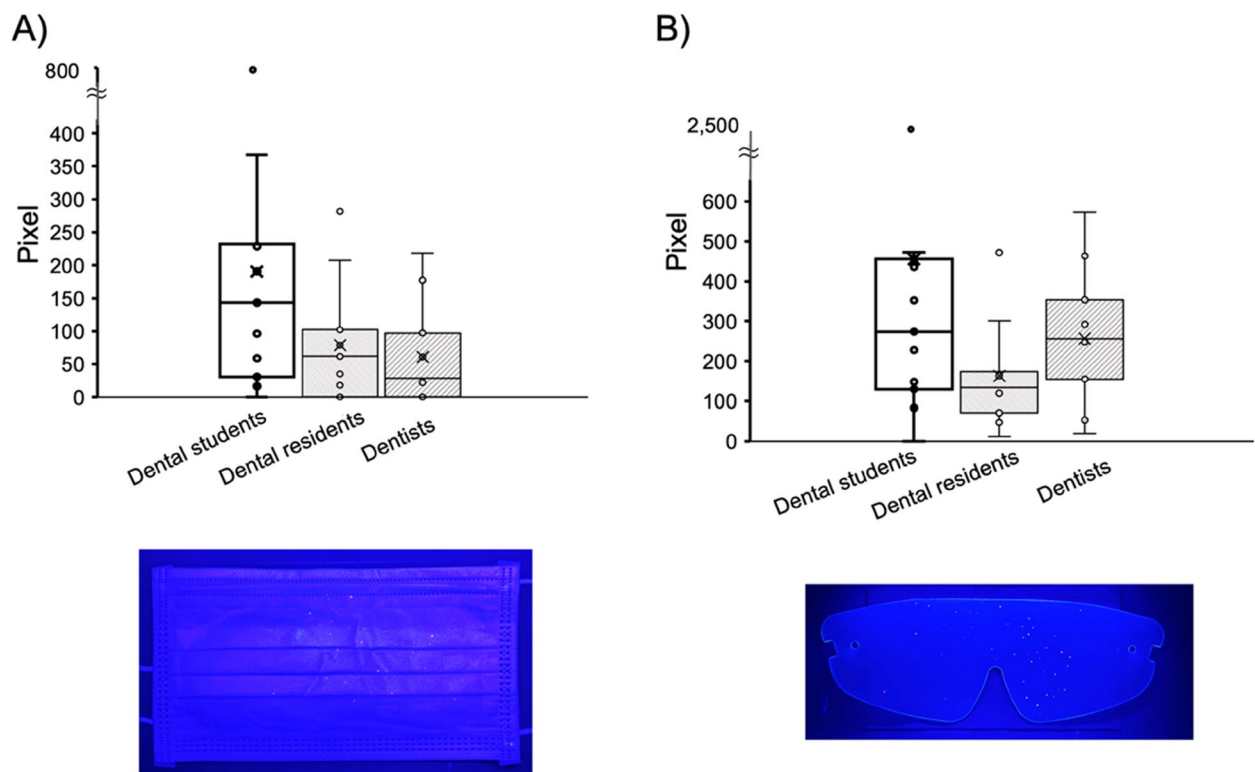


Fig. 6 Fluorescent areas on PPE. The average fluorescent areas measured by two blinded evaluators using ImageJ software® are shown. Data from the three groups (10 participants each from dentists, dental residents, and dental students) are analyzed using the Kruskal-Wallis test. **A** Fluorescent areas on the surface of the surgical mask: There are no significant differences between the groups. A typical image is presented in Figure. **B** Fluorescent areas on the surface of the face shield: There are no significant differences between the groups. A typical image is presented in Figure

Table 1 Numbers of participants with positive fluorescent areas

	Dental students	Dental residents	Dentists	P value
Desk	8	9	10	0.329
Operators' chair	5	8	2	0.034*
Surgical cap	4	6	1	0.065
Plastic apron	9	9	10	0.585
Pencil	10	4	9	0.006*

Number of people out of 10 (10 participants each)
 Fisher's exact test
 *P < 0.05

lotion, similar to yogurt only. This mixture also allowed for the visualization of the droplets and directly touched areas. The measurement of *Lactobacillus* ATP levels in yogurt has been previously reported [25]; however, its application in dental education for infection control has never been reported to our knowledge. The results of this study indicate the possibility of applying the newly

devised artificial saliva in preclinical dental practice with dental phantoms.

This trial for clinical education was conducted to investigate whether preclinical practice using artificial saliva, a mixture of yogurt and hand lotion, would be helpful for student education on infection control skills in preclinical curricula. Our results demonstrate that *Lactobacillus* ATP levels in yogurt could be a marker of infectious amounts around dental phantoms in preclinical student practice. Additionally, the mixture revealed by UV light enabled us to visualize areas directly touched by the hands, with gloves and droplets attached to the surroundings around the dental equipment. The evaluation of infectious areas based on the measurement of ATP levels showed both operability and reproducibility. Periods of experience as a dentist were significantly related to ATP levels on surgical masks and face shields during infection control operations. This result is similar to the fact that PPE use compliance was associated with age, years of practice, medical institution

type, and exposure risk estimation [15]. However, our study observed relatively young dentists (an average of 3.2 years) because we evaluated the educational effects on graduate students (the fifth-grade Japanese dental school) and dental residents six months after obtaining Dental License.

In contrast, luminous areas were sometimes ambiguous and unjudgeable, with less correlation with visual fluorescence intensity in some cases. Therefore, not only the luminous areas and presence clarified with hand lotion but also the combination with the measurement of ATP level implements the evaluation of precise infectious degrees in preclinical education. This quantitative evaluation method for infectious areas and degrees was also used to expose droplet ranges, particularly when flossing. This observation is the first report we have heard. The droplet ranges did not depend on the experience duration, indicating the importance of PPE. Thus, this evaluation system of the degree of infection using *Lactobacillus* ATP and UV-sensitive fluorescent hand lotions is worthy of preclinical dental education. Its utilization would improve operators' awareness and modify their behavior for infection control.

A limitation of this study is that the practice content must be limited to practice and examination without water and air blowing. The mixture of yogurt and hand lotions used as artificial saliva is white and turbid, with the possibility of being discerned by operators. Future investigations in multiple facilities with expanded eligibility are required.

Conclusions

This study established a system to evaluate infection control in preclinical dental practice using *Lactobacillus* ATP and a UV-sensitive fluorescent hand lotion. The system identified infectious areas and degrees of infection during preclinical practice using a dental phantom. The degree of infection of personal equipment is related to the duration of experience as a dental practitioner. This system to evaluate infection control would be beneficial in an accurate, rapid, and inexpensive way for preclinical dental practice.

Abbreviations

ATP	Adenosine triphosphate
COVID-19	Coronavirus disease 2019
LED	Light emitting diode
PPE	Personal protective equipment
PBS	Phosphate buffer solution
RLU	Relative light unit
SD	Standard deviation
UV	Ultraviolet

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12903-025-05584-4>.

Supplementary Material 1

Acknowledgements

The authors would like to express their gratitude to all the participants.

Authors' contributions

AU contributed to investigation, data curation and visualization. YSI contributed to conceptualization, and writing original draft. KTH contributed to conceptualization and data curation. TI supervised the design of the clinical education trial. SO helped to perform the trial for clinical education. KH contributed to methodology and the recruitment of participants. KO supervised this study. TY contributed to the recruitment of participants and supervised this study. ST contributed to conceptualization, methodology and supervision.

Funding

This study received no funding.

Data availability

Data will be made available on request.

Declarations

Ethics approval and consent to participate

This study was approved by the Okayama University Ethics Committee (Approval No. 2308-032). Informed consent was obtained from all the participants for this study.

Consent for publication

The written informed consent was obtained from all the participants to publish the data on this study.

Competing interests

The authors declare no competing interests.

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Received: 3 July 2024 Accepted: 30 January 2025

Published online: 08 February 2025

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