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Research Article

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Real-Time Feedback of ATP Bioluminescence in Evaluating the Handwashing Efficiency of Healthcare Personnel

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Abstract

Background: Providing prompt, real-time feedback on hygiene status plays a critical role in on-site supervision. Therefore, research into using ATP bioluminescence for real-time hand hygiene assessment is of great importance during public health emergencies.

Methods: ATP bioluminescence assays and aerobic culture tests were conducted to assess the hand hygiene status of 13 healthcare workers. Hand samples were collected from the participants before handwashing, after handwashing with tap water and after handwashing with antibacterial liquid soap. The reductions in ATP RLU and aerobic colony counts were evaluated for each handwashing stage.

Results: Following handwashing with tap water, the reduction ratio of ATP RLU and the inactivation ratio of aerobic cultures on hands were 68.39% and 59.50%, respectively. After handwashing with antibacterial hand-soap, the ratios increased to 92.49% and 80.86%, respectively. The handwashing efficacy of antibacterial soap was significantly superior to that of handwashing with tap water alone (ATP RLU P<0.001, aerobic cultures P=0.001).

Conclusion: The reduction ratios derived from ATP bioluminescence detection and aerobic cultures objectively reflect the effectiveness of hand hygiene. ATP bioluminescence detection may serve as an auxiliary tool for on-site sanitation supervision.

Keywords: ATP; Aerobic culture; Hand hygiene; Disinfection; Detection

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Introduction

Hand hygiene and disinfection quality of environmental surfaces serve as core links to block contact transmission of pathogenic microorganisms and reduce the risk of cross-infection and this importance is prominent in public venues such as medical institutions, elderly care facilities, kindergartens and hotels [1-4].

Healthcare professionals, such as laboratory sampling and testing personnel, are the core workforce responsible for inspection and sample analysis. Their hand hygiene compliance is directly associated with the accuracy of experimental results, biosafety management and the prevention of cross-contamination. Compared with medical staff in healthcare facilities, laboratory personnel have relatively lower awareness and compliance with hand hygiene requirements [5]. Inadequate hand hygiene and disinfection may lead to cross-contamination of samples, data inaccuracies and biosafety risks, posing a threat to the health of staff [6]. Therefore, conducting regular and accurate monitoring of hand hygiene and disinfection quality is critical for standardizing laboratory operations, guaranteeing testing quality and improving

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laboratory biosafety [7].

Adenosine Triphosphate (ATP) bioluminescence detection has become a core method for addressing the limitations of traditional detection methods, owing to its unique advantages including ultra-rapid detection, quantifiable measurement results, simple operation and suitability for high-throughput screening. Based on the specific luminescence reaction catalyzed by firefly luciferase for ATP, this technology measures the Relative Light Unit (RLU) readings of samples through portable professional detectors, calculates the residual ATP level on surfaces and indirectly evaluates microbial contamination and disinfection efficacy. Relevant reports indicate that reduction in ATP RLU in disinfection with alcohol pads, alcohol-based hand rub and hydrogen peroxide disinfectant wipes are equivalent in Colony Forming Unit (CFU) reduction [10]. ATP testing has been well-established for application in the medical and health field [11,12]. It is capable of reflecting the levels of organic matter and microbial load on the hands of medical personnel and environmental surfaces and provides quantitative evidence for the evaluation of disinfection efficacy [13-15]. This method can reflect both the organic matter content and microbial load levels on the hands of medical personnel and can provide a quantitative basis for evaluating the disinfection efficacy.

In this study, the hand hygiene of healthcare personnel was chosen as the research subject. The ATP bioluminescence assay and aerobic cultures were concurrently employed for sample detection to analyze their correlation and practical applicability.

Methods

Ethics approval

According to the Ethics Committee of Guangxi Center for Disease Control and Prevention, ethical review was waived because the study involved routine mandatory hand hygiene surveillance conducted by public health administrative authorities, with non-invasive sampling, anonymized data collection and no experimental intervention or identifiable participant information. Every participant was fully informed of the sampling procedure and purpose and verbal cooperation consent was obtained prior to sampling.

Sampling and detection of ATP RLU

A portable ATP bioluminescence detector (BIOLUM, Tianlong Science & Technology, Xi'an, China) and its corresponding ATP bioluminescence test swabs were used. First, incubate the swabs at room temperature for 10-20 min, unscrew the reaction tube located at the base of the swab. Wipe the testing surface with the cotton swab tip at an angle of 15°-30° and swab horizontally and vertically 5 times while rotating the swab. Second, recap the reaction tube onto the swab, remove the upper protective cap of the swab, hold the assembly vertically and press the spring cap repeatedly to draw all the reagent into the reaction tube. Next, hold the upper spring cap and oscillate the swab laterally with an approximate 30° swing for 5 s to achieve thorough mixing. Finally, insert the prepared swab into the test chamber of the ATP detector on the standby interface and close the lid to start the measurement. Each sample should be measured in triplicate within 1 min and the average value should be calculated. Calculate the ATP RLU reduction ratio of each sample as follows: (RLU of the sample before treatment - RLU of the sample after treatment) ÷ RLU of the sample before treatment × 100%.

Sampling and detection of aerobic cultures from hands

Sampling was conducted on the hands of healthcare personnel for hygiene surveillance before and after the implementation of hand hygiene protocols. The left hand was designated for the detection of ATP, while the right hand was utilized for the purpose of aerobic culture testing. Sterile Phosphate Buffered Saline (PBS) was employed for the purpose of pre-wash hand sampling. PBS containing a neutralizer (0.5% Tween 80+0.2% sodium thiosulfate+0.1% lecithin) was applied for sampling after handwashing with water and after handwashing with antibacterial hand soap, respectively. Sterile swabs were then moistened with the corresponding solution and used to wipe the flexor surfaces and palms of all fingers on each hand back and forth twice. Subsequently, the portion of the swab shaft that had come into contact with the hands was excised and the swab tips were transferred into a test tube containing 10 mL of sampling solution. Following the vortexing of the solution, 1.0 mL of the eluate was inoculated onto nutrient agar and incubated at 36°C for 48h. The colony reduction ratio of each sample was calculated as follows: (CFU per sample before treatment - CFU per sample after treatment) ÷ CFU per sample before treatment × 100%.

Data processing and statistical analysis

The data on ATP RLU and CFU were imported into WPS Excel (Kingsoft, Beijing, China) and GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). The Wilcoxon test was employed to analyze the reduction ratio of ATP, RLU and CFU for each group after cleaning and disinfection. Spearman's correlation analysis was conducted to evaluate the correlation between ATP RLU and CFU. Statistical significance was defined as a P-value less than 0.05.

Results

ATP RLU reduction

The ATP RLU detected on hand surfaces prior to any treatment ranged from 166 to 7381 per hand with a calculated median of 2086 per hand. Following the completion of standard handwashing procedures with tap

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water, the elimination ratio of ATP RLU on hand surfaces was 68.39%. By contrast, after handwashing with antibacterial soap using the standard procedure, the reduction in ATP RLU compared to the untreated baseline was 92.49% **(Table 1)**.

Aerobic colonies reduction

The aerobic colony counts recovered from the surfaces of tested hands before any disinfection intervention ranged widely from 170 to 30,000 CFU per hand with a calculated median value of 6,400 CFU per hand among all tested samples. In this study, the elimination ratio of aerobic colonies obtained through different handwashing methods was found to be lower than the corresponding reduction ratio of ATP RLU. When compared with the aerobic colony count status measured before any handwashing intervention, the elimination ratio of aerobic colonies reached 59.50% after simply handwashing with tap water and the elimination ratio rose to 92.49% after standardized handwashing with commercial antibacterial hand soap **(Table 1)**.

The efficacy of handwashing with tap water only or with antibacterial soap in reducing residual ATP, RLU and CFU on the hands has been well documented. Nevertheless, in comparison with handwashing using tap water, antibacterial hand soap achieves superior removal of microbial contaminants and delivers enhanced cleaning and bacteriostatic efficacy.

Table 1: Results of hand hygiene (n=13).

Item	Before handwashing		After handwashing with tap water			After handwashing with antibacterial hand soap		
	Median	Range	Median	Range	Reduction ratio (%)	Median	Range	Reduction ratio (%)
ATP RLU	2086	166-7381	421	45-1715	68.39	70	14-542	92.49
CFU	6400	170-30000	800	100-12000	59.50	200	60-2800	80.86

A paired Wilcoxon signed-rank test was employed to analyze the reduction ratios of ATP, RLU and CFU subsequent to handwashing with either tap water or antibacterial hand soap. The findings indicated that the reduction ratio of ATP RLU subsequent to handwashing with antibacterial hand soap was significantly greater than that following tap water washing (P<0.001). Furthermore, the reduction ratio of CFU was also significantly increased in the antibacterial soap group (P=0.001). Furthermore, an evaluation of the correlation between ATP RLU and CFU by Spearman's rank-order correlation analysis revealed that there was no significant correlation between ATP RLU and CFU prior to handwashing (r=0.259, P=0.393). A significant positive correlation was observed between ATP RLU and CFU (r=0.606, P=0.028) subsequent to handwashing with tap water, suggesting that a higher ATP RLU was associated with a greater number of aerobic cultures. However, no statistically significant correlation was identified between ATP RLU and CFU following handwashing with antibacterial hand soap (r=0.538, P=0.058).

Discussion

The present study employed ATP bioluminescence detection and conventional aerobic cultures to assess the hand hygiene of healthcare workers. The evaluation of hand hygiene revealed that the practice of hand-washing with tap water and antibacterial hand soap led to a significant reduction in both residual ATP levels and microbial loads. The reductions in ATP RLU and CFU were consistent after handwashing.

Following the implementation of a handwashing regime with antibacterial soap, a significant decline in ATP, RLU and microbial ratios was observed. The reduction ratios achieved 92.49% and 80.86%, respectively, which were substantially higher than the values recorded for handwashing with tap water, which were 68.39% and 59.50%, respectively. The efficacy of handwashing with tap water is contingent on the presence of physical flushing, a process which is capable of removing superficial contaminants and transient flora from the skin. However, this method is ineffective in the elimination of stubborn organic substances encapsulated in skin sebum and resident microorganisms. Conversely, antibacterial hand soap has been demonstrated to be efficacious in the dissolution of sebum and organic residues on the hands, in addition to the inactivation of both resident and transient skin bacteria. This results in a more comprehensive decontamination and bactericidal effect. The utilization of alcohol-based hand rub for the purpose of hand hygiene represents the most prevalent approach in the field of hand hygiene. However, the disinfection of hands with 75% alcohol led to a reduction in aerobic cultures, while increasing the values of ATP RLU in our exploratory experiments. A hypothesis that may be worthy of consideration is that alcohol has the capacity to dissolve and emulsify organic substances, thereby resulting in a substantial release of ATP from the deeper layers of the skin to the surface. This has been shown to enhance the extraction efficiency of sampling swabs and the assay response, thereby causing a spurious elevation of RLU readings. This phenomenon does not signify a genuine resurgence of contamination attributable to suboptimal disinfection efficacy. When utilizing the ATP assay for the purpose of hand hygiene assessment subsequent to alcohol disinfection, particular consideration may be devoted to this issue.

Correlation analysis indicated that the correlation between hand ATP RLU and CFU exhibited distinct stage-

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specific features, which serves as a crucial interfering factor that is easily neglected during the practical implementation of rapid ATP detection. There was no significant correlation between hand ATP RLU and CFU at the baseline prior to handwashing (P=0.393). As reported by Vaca González, Juan Jairo, Surgical soap demonstrated a statistically significant difference in the reduction of ATP RLU, yet no such difference was observed in CFU [18]. The fundamental cause lies in the complex composition of the initial contaminants on human hands. ATP signals come not only from bacterial cells but also from a large amount of non-bacterial organic matter, including skin metabolites, environmental dust and exogenous dirt. This means ATP readings cannot accurately reflect the actual viable bacterial load. After handwashing with tap water, most non-bacterial miscellaneous organic substances are washed away and the residual ATP on the skin is mainly derived from microbial cells. This leads to a significant positive correlation between ATP RLU and CFU (P = 0.028). At this stage, ATP measurement can reliably indicate the degree of bacterial contamination on hands.

This study has certain limitations. First, the sample size was limited and no comparative analysis was conducted between ATP readings and colony counts for hands treated with different types of disinfectants. Furthermore, due to constrained funding and an insufficient supply of ATP swabs, the interference mechanism of handwashing soaps and disinfectants on ATP signals and the related influencing factors have not been systematically clarified. We will conduct further in-depth exploration of these aspects in future studies.

In summary, the ATP bioluminescence assay is rapid and convenient, enabling routine and prompt screening for hand hygiene. Nevertheless, it is susceptible to interference and may lead to disparities between ATP readings and counts of viable microorganisms at specific stages. ATP bioluminescence detection may serve as an auxiliary tool for on-site sanitation supervision.

Author Contributions

Chunai Tao: Formal analysis, investigation, methodology, project administration, resources, supervision, visualization and writing-original draft.

Shanfei Yan: Data curation, investigation, writing-review and editing.

Jing Qin: Investigation, software, writing, review and editing.

Zefei Chen: Investigation.

Guining Lu: Investigation and supervision.

Yongxin Gan: Conceptualization, methodology, project administration, supervision, validation and visualization.

Conflict of Interest Statement

The authors declare no conflicts of interest.

Funding Declaration

The authors declare no founding resources.

Data Availability

The datasets used and analyzed during the current study can be available from the corresponding author on reasonable request.

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