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Research Article

Evaluation of ATP Bioluminescence Compared to Colony Counting Method for Measuring Cleanliness and Disinfection Efficiency of Different Surfaces

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Abstract

Prompt evaluation of cleanliness and disinfection efficacy is significant for on-site scenarios. Adenosine triphosphate (ATP) bioluminescence assay is effective for hygiene evaluation, while the ATP relative light unit (RLU) differs from various surfaces. This study compared the effectiveness of ATP bioluminescence assay and colony counting method in evaluating the cleanliness and disinfection efficiency on different surfaces, and to explore the feasibility of on-site ATP evaluation. Evaluation of disinfection efficiency was conducted in the laboratory floor by disinfecting 900 mg/L chlorine disinfectant for 30 min, and the ATP RLU and the total bacterial colonies were detected before and after disinfection. On-site sampling and detection of ATP and total bacterial colonies were performed on the surface and floor of the food processing workshop. Following the disinfection with chlorine disinfectant, the killing ratio of natural bacterial colonies and the elimination ratio of ATP RLU decreased by 90.52% and 86.87% respectively. Spearman rank-correlation analysis indicated that ATP RLU exhibited a positive correlation with colony forming unit (CFU). The ATP RLU of the tables and floors in the workshop were within the ranges of (14 - 849)/100 cm² and (131 - 8437)/100 cm² respectively, and the total bacterial colonies were within the ranges of (0 - 200) CFU/100 cm² and (30 - 800) CFU/100 cm² respectively. Pearson correlation analysis demonstrated that there was no linear correlation between Log RLU and Log CFU on the tables, whereas a linear positive correlation was detected for the floor samples. The decrease ratio of ATP RLU was consistent to the total colony count for disinfection, but the relationship was intricate in the cleanliness evaluation within the workshop. The two methods may be integrated comprehensively to evaluate the cleanliness and disinfection of surface.

Keywords

ATP, Relative Light Intensity, Cleanliness, Disinfection, Evaluation

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1. Introduction

Disinfection refers to the process of eradicating pathogenic microorganisms from the environment [1]. The disinfection process is affected by factors like disinfectant, concentration, time, temperature, humidity, organic matter, and microorganism. Inadequate disinfection in medical settings may lead to hospital - acquired infections and endanger human health. Scientific evaluation can determine disinfection effectiveness, preventing pathogen persistence from incomplete disinfection and avoiding infectious disease outbreaks [2].

Cleaning and disinfection evaluation helps identify and correct violations, ensuring responsible parties to obey the obligations and also boosts public health awareness and trust. Currently, the colony culture counting method remains the 'gold standard' for microbial contamination, but it is time-consuming, typically lasts 24-48h. ATP bioluminescence, a rapid detection method, has gained attention in fields of food hygiene inspection [3], medical diagnosis, environmental monitoring and biopharmaceuticals, and animal husbandry fields [4-7]. ATP serves as an energy supplier in all living cells. It is transformed into adenosine diphosphate through chemical or light-mediated processes to release energy and sustain cellular metabolism. As a health indicator, the level of ATP RLU reflects the biological load. The ATP detection technique employs luciferase to catalyze the oxidation of fluorescein, thereby generating fluorescence. The luminescence value is measured by a fluorescence detector, enabling the determination of ATP RLU and bacterial load [8].

The ATP detection method is an effective tool for hygiene evaluation. It provides immediate, numerical pollution assessment, allowing for on-site identification and timely feedback [9, 10]. However, its accuracy and reliability are influenced by various factors such as sampling methods, the presence of interfering substances, and the sensitivity of the detection instrument. This study aims to systematically evaluate the performance of the ATP detection method in environmental surface disinfection assessment, and compare it with the traditional colony culture counting method to explore its feasibility, advantages, and limitations in practical monitoring scenarios. Specifically, we will analyze the correlation between ATP RLU and bacterial colony counts. The findings of this research are expected to provide a scientific basis for the application of ATP bioluminescence technology in improving the quality control of environmental cleaning and disinfection.

2. Methods

2.1. ATP Sampling and Detection

ATP sampling and detection was conducted with a portable ATP fluorescence detector (Xi'an Tianlong, Biolum, China) and its ATP bioluminescence detection swab. The procedures included sampling, pyrolysis, reaction, and data acquisition. A cotton swab was employed to sample the surface with a 5×5 cm sterile plate at an angle ranging from 15° to 30°and cover for 100 cm². Simultaneously, the swab was rotated five times both horizontally and vertically. Next, the reaction tube was installed, and the reagent was injected by pressing the lid. The swab was then mixed with the reagent, followed by shaking the tube at a 30° angle for 5s. After closing the lid to initiate the process, 3 repeated tests were conducted within 1 min, and the average value was recorded. The tests were conduced at 20–22°C.

2.2. Sampling and Detection of Natural Bacterial Colonies on Surfaces

A 5×5cm sterile plate and a cotton swab soaked in neutralizer (0.3%lecithin+0.3%sodium thiosulfate+0.3%glycine+1%Tween-80) solution were utilized for sampling. The swab was smeared five times horizontally and vertically on the plate, and subsequently rotated to cover an area of 100 cm². The swab was then placed into a test tube containing 10 mL of sampling solution. After shaking, 1.0 mL of the eluent was inoculated onto a plate with nutrient agar, and the plate was incubated at 37°C for 48 h. The total number of bacterial colonies was counted, and calculated in terms of CFU/cm².

2.3. Assessment of Disinfection Efficiency

The experimental was conducted on the floors in primary and secondary laboratories. The area had not cleaned for two months, resulting in the accumulation of organic matter, dust, and microorganism. ATP RLU and natural bacterial colonies were collected before and after disinfection respectively. Chlorine disinfectant tablets (containing 45% sodium dichloroisocyanurate, DCCNa) were utilized to prepare 900mg/L solutions in accordance with the manufacturer's specifications. The disinfection entailed spraying at a rate of 0.7 L/min and a dosage of 200 mL/m² until the surfaces were moistened. Samples were collected 30 min after spraying. The ATP RLU elimination ratio was computed as (ATP RLU before - ATP RLU after) / ATP RLU before × 100%, and the killing ratio of natural bacterial colonies was computed as (colonies before - colonies after)/colonies before× 100%.

2.4. Evaluation of Cleanliness and Disinfection Efficiency in Workshop

The hygiene of tables and floors in the food processing workshop were chosen for the evaluation of cleanliness and disinfection. The tables were stainless steel and the floor was coated with smooth floor paint. After the daily cleaning process, the tables were wiped and disinfected using 75% food - grade alcohol, whereas the floors were disinfected with 200 mg/L of sodium hypochlorite disinfectant. Sampling was carried out in two areas. One was the operating area, the other

area was cleaned and disinfected and left undisturbed overnight (approximately 16 hours).

2.5. Data Processing and Analysis

The ATP RLU and the total bacterial colonies were imported into WPS Excel (Beijing Jinshan, China) and GraphPad Prism 6.0 (GraphPad, San Diego, CA, USA) and analyzed using the Spearman rank and Pearson correlation test. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Evaluation of Disinfection Efficiency

The ATP RLU of the floor before disinfection was within the range of (7-1688)/100 cm², with a median 502.8/100 cm², and was within (2-229)/100 cm², with a median 21.3/100 cm² after disinfection. The mean elimination ratio was 86.87% (Figure 1). Besides, negative control of ATP RLU by testing the 900mg/L chlorine disinfectant was 0. The total number of natural bacterial colonies on the floor before disinfection was within the range of (4-740) CFU/100 cm², median 114.0 CFU/100 cm² and was within the range of (0-190) CFU/100 cm², median 3.0 CFU/100 cm² after disinfection (Figure 2). The killing ratio of the total bacterial colonies was 90.52%. The ATP RLU was comparable to the total number of colonies.

As the ATP RLU and CFU were skewed distributed, they were tested for normality after logarithmic transformation, and $P<0.05$ for log-RLU and log CFU, respectively, which did not conform to normality. Therefore, Spearman rank correlation analysis was used. Analysis of ATP RLU and CFU before disinfection demonstrated that the ATP RLU displayed a positive correlation with CFU (r=0.490, P =0.028). This implies that a higher ATP value was associated with a higher CFU. Additionally, Spearman rank correlation analysis of ATP RLU and CFU after disinfection indicated that there was no significant correlation between ATP RLU and CFU (r=0.318, P=0.172), which failed to explain the monotonic correlation between the two variables.

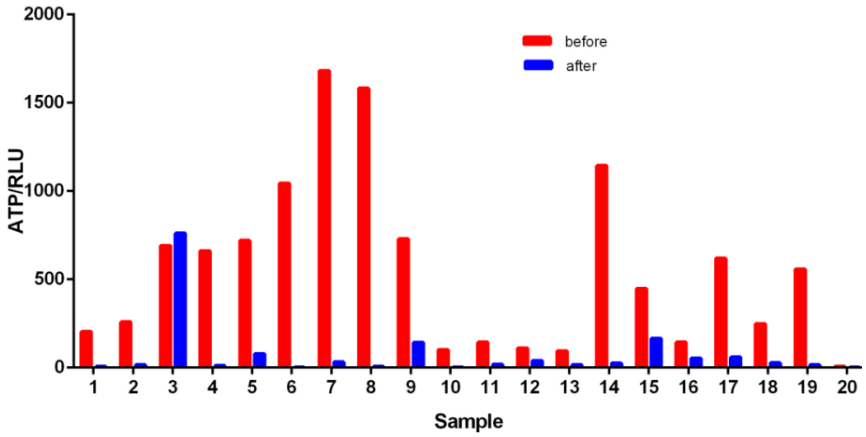

Figure 1. ATP RLU before and after disinfection.

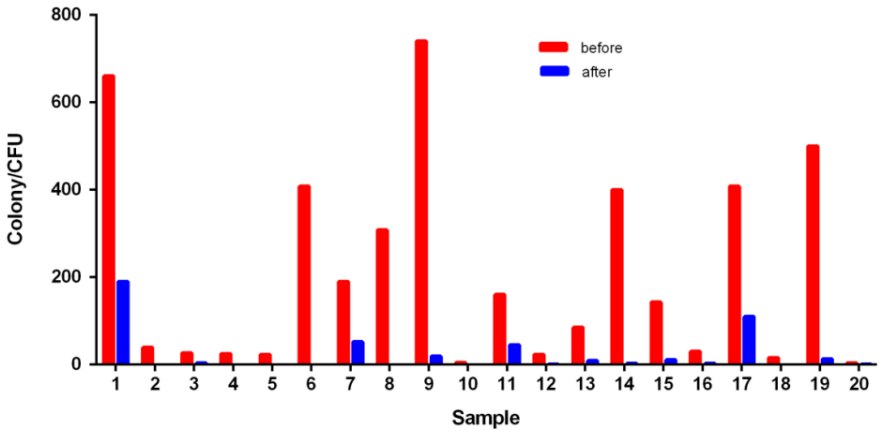

Figure 2. Colony numbers before and after disinfection.

3.2. Assessment of the Cleanliness and Disinfection Efficiency in Workshop

Tables: The sampling of the food processing workshop was classified into the operating area (Samples 1-10) and the area left overnight after cleaning and disinfection (Samples 11-20). The ATP RLU of the tables was within the range of (14 - 849)/100 cm², with a median 51/100 cm², and the total number of colonies was within the range of (0-200) CFU/100 cm², with a median 14 CFU/100 cm² (Figure 3).

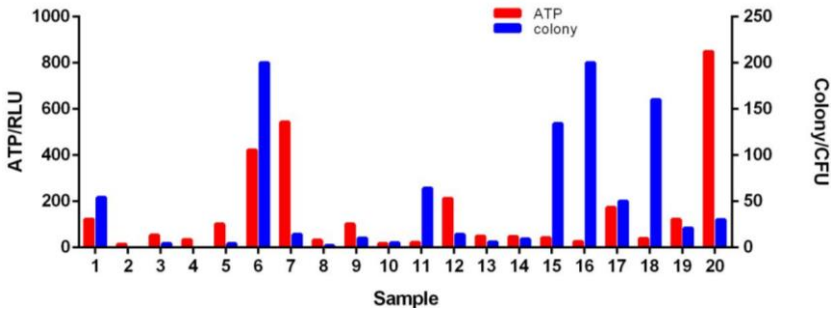

Figure 3. *ATP RLU and colony numbers for tables. Samples 1-10 are operating area, 11-20 are area left overnight subsequent to cleaning and disinfection.*

Table samples 11-20 were from the area left overnight subsequent to cleaning and disinfection. ATP testing revealed that the majority of the values were low; however, the number of total bacterial colonies above 200 CFU/100 cm^2 were slightly higher than the operational area. It is evident that despite the cleaning and disinfection of the operating tables after utilization, microorganisms still proliferated overnight, leading to the re-contamination of the tables. It is essential to carry out another round of cleaning and disinfection before working.

As the ATP RLU and CFU for tables were skewed distributed, the normality test was performed after logarithmic transformation, and $P > 0.05$, respectively. Therefore, Pearson correlation analysis was used to analyze the correlation, and revealed that the correlation coefficient between Log RLU and Log CFU was not statistically significant (r=0.288, P=0.217), suggesting that there was no linear correlation between the two tables variables.

Floors: Floor samples 1-10 were in the operating area, samples 11-20 were in the area after cleaning and disinfection. The ATP RLU of the floors was within the range of (131 - 8437)/100 cm², with a median 904.0/100 cm², and the total number of colonies was within the range of (30-800) CFU/100 cm², with a median 306.0 CFU/100 cm² (Figure 4). The ATP RLU and total number of colonies were higher in the floor of the operating area. It can be seen that the ATP RLU was lower after cleaning and disinfection, and the total number of colonies was also lower than that of the floors in the operating area. This implied that the floor remained clean without contamination by personnel and items.

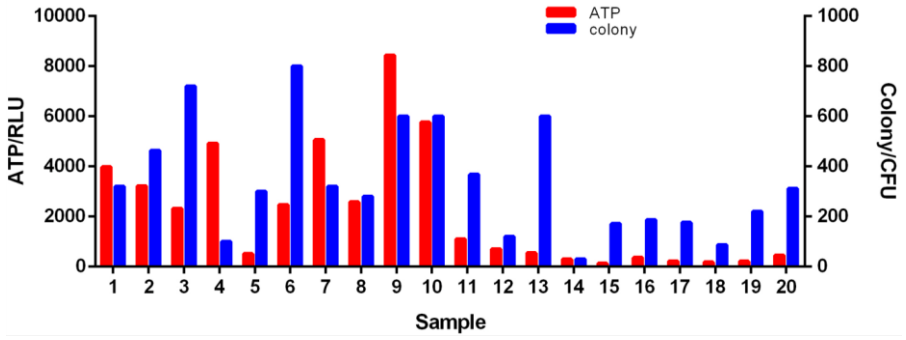

Figure 4. *ATP RLU and colony numbers for floor. Samples 1-10 are operating area, 11-20 are area left overnight subsequent to cleaning and disinfection.*

As the ATP RLU and CFU for floors were skewed distributed, the normality test was performed after logarithmic transformation, and $P > 0.05$, respectively. Therefore, Pearson correlation analysis was applied. It showed that the log RLU was positively correlated with the log CFU of the floor, and the difference was statistically significant (r= 0.530, P= 0.016), suggesting a linear positive correlation between the two floor groups.

4. Discussion

This research evaluated the disinfection efficacy on laboratory floors using ATP detection and colony counting methods, along with assessing cleanliness and disinfection effectiveness in the workshop. It showed that the decrease ratio of ATP RLU was consistent to the total colony count for disinfection, but the relationship was intricate in the cleanliness evaluation within the workshop.

In accordance with the evaluation criteria for on-site disinfection efficacy (WS/T - 2022) [11] in China, a killing ratio of natural bacteria colonies more than 90% on the surface is considered qualified after disinfection. Considering the organic matter, mould, and dust on the surface of the floor, 900 mg/L sodium dichloroisocyanurate and a residual time of 30min was employed for disinfection. The killing ratio of natural bacterial and mould colonies was 90.2% after disinfection, indicating a qualified disinfection. The ATP detection revealed that the elimination ratio of RLU was 86.87%, suggesting it also had a favorable disinfection effect. Moreover, surfaces rich in organic matter usually have a higher ATP level, but the colony count is not definitely high. In order to rule out the influence of chlorine disinfectant on ATP fluorescence detection values, the ATP values of disinfectants were measured independently, and no values were detected in this study. ATP RLU detection might be useful for evaluating surface disinfection.

The assessment of the cleanliness level and disinfection efficiency within the workshop indicates that there was no linear correlation between the ATP RLU and the natural colonies detected on the tables. That is, as the ATP RLU varied, the quantity of natural colonies on the tables did not exhibit a proportional increase or decrease. Conversely, in the case of the floor samples, a distinct linear positive correlation was observed, which suggests that as the ATP RLU values of the floor samples increased, the quantity of natural colonies also increased, indicating there is a more predictable connection between these two factors for the floor samples than for the tables. The ATP bioluminescence method is affected by numerous factors such as pH, temperature, enzyme concentration, and luminescence determination time. A substantial number of experiments are required to establish the optimal conditions for the reaction system [6]. Establishing the optimal conditions for the ATP reaction system is challenging. Although ATP provides quantitative data, its accuracy can be affected by the presence of detergents or disinfectants, it still necessitates careful calibration.

Studies have demonstrated that ATP detection has gained popularity and is increasingly utilized to assess hygiene effectiveness in many fields [12]. The complexity of microorganism and organic matter on the surface adds to the difficulty of quantitatively comparison of ATP RLU and colony counting methods. However, researchers propose that a semi-quantitative detection limit can be determined from much test data. Test standards like qualified, warning, and unqualified can be set by analyzing results and considering industry health requirements [12]. The ATP fluorescence method is effective for evaluating environmental cleaning efficacy [13, 14]. Research shows it can be used in hospital cleaning and disinfection, evaluating surface, device, endoscope cleaning, and staff hand hygiene [15, 16], also playing supervisory and educational roles. But due to many influencing factors, the evaluation criteria are not still unified [17], so it can not thoroughly replace the traditional colony counting method.

5. Conclusion

In conclusion, ATP monitoring is a quick and easy way to assess contamination and cleanliness, and can serve as a preliminary assessment for the rapid evaluation of the cleanliness and disinfection efficacy of surfaces. The two methods may be integrated comprehensively to evaluate the pollution and disinfection of surface and prepare for early warning.

Abbreviations

ATP	Adenosine Triphosphate
RLU	Relative Light Unit
CFU	Colony Forming Unit
DCCNa	Sodium Dichloroisocyanurate

Author Contributions

Chunai Tao: Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft

Xiaoying Qin: Data curation, Investigation, Software, Writing – review & editing

Chunfei Wan: Investigation, Investigation, Software, Writing – review & editing

Yongxin Gan: Conceptualization, Methodology, Project administration, Supervision, Validation, Visualization

Xianke Liang: Funding acquisition, Resources

Conflicts of Interest

The authors declare no conflicts of interest.

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