Sep 07, 2019

Paired design of ATP bioluminescence method and colony counting method

PLOS One

Huiqiong XU; Jiansheng LIANG; Yimei WANG; Bin WANG; Tianbao ZHANG; Xiaoli LIU1; Lin GONG[1]

[1]Wuhan Centers for Disease Prevention and Control, Wuhan, Hubei, China

1 Works for me dx.doi.org/10.17504/protocols.io.49hgz36

Huiqiong Xu
Wuhan Centers for Disease Prevention and Control

ABSTRACT

The ATP bioluminescence method can detect the ATP content in a sample by monitoring the biological luminescence reaction of a luciferase assay with a luminometer to detect the presence of microorganisms or other organic residues indirectly.Some studies demonstrated a good correlation between the ATP bioluminescence method and the colony counting method. However, due to the absence of uniform evaluation standards, most medical institutions rely on the reference values provided by the manufacturer of the testing detectors. And different detectors have different recommended values.Therefore, our study aimed to compare the ATP bioluminescence method with the colony counting method and the ATP bioluminescence method , also compare different ATP bioluminescence detector types, for monitoring the disinfection effect of environmental surfaces.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0221665

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Xu H, Liang J, Wang Y, Wang B, Zhang T, Liu X, Gong L (2019) Evaluation of different detector types in measurement of ATP bioluminescence compared to colony counting method for measuring bacterial burden of hospital surfaces. PLoS ONE 14(9): e0221665. doi: 10.1371/journal.pone.0221665

Laboratory Protocols.docx

MATERIALS TEXT

Device: B (BT-112D, Beijing Chuangxin Shiji Biochemical Science&Technology Development Co.,Ltd., China), P (SystemSURE Plus, Hygiena, USA), and N (Clean-Trace NGi, 3M, USA), pipette,Incubator

Reagent: ATP standard solution (The concentration of ATP standard solution should be no less than 1.0×10^{-7} mol/L), pure water, the Clean-Trace™ ATP Surface Test (suitable for N, 3M, USA), the UltraSnap™ATP Surface Test (suitable for B and P, Hygiena, USA), Nutrient agar medium, Sterilized test tube containing a neutralizer (0.1% sodium thiosulfate)

1 Prepare the standard curve

Device: B (BT-112D, Beijing Chuangxin Shiji Biochemical Science&Technology Development Co.,Ltd., China), P (SystemSURE Plus, Hygiena, USA), and N (Clean-Trace NGi, 3M, USA), pipette

Reagent: ATP standard solution (The concentration of ATP standard solution should be no less than 1.0×10^{-7} mol/L), pure water, the Clean-Trace^{TM} ATP Surface Test (suitable for N, 3M, USA), the UltraSnap^{TM}ATP Surface Test (suitable for B and P, Hygiena, USA)

First, the ATP standard solution was serially diluted in pure water to obtain the following concentrations:1.0×10^{-7}mol/L, 5.0×10^{-8} mol/L, 1.0×10^{-8} mol/L, 5.0×10^{-9}mol/L, 1.0×10^{-9} mol/L, 5.0×10^{-10} mol/L, 1.0×10^{-10}mol/L, 5.0×10^{-11} mol/L, 1.0×10^{-11} mol/L, 5.0×10^{-12}mol/L, and 1.0×10^{-12} mol/L.

Two ATP surface test sticks were used for the detectors involving a swab and some reagents. Second, Aliquots of 10 µL were removed from each dilution and dropped onto the swab by pipette, and mix the swab with the reagents of the surface test stick, shaking 20 times. Then, put the surface test stick into the detector (B, P or N) detection chamber and obtain the RLU value according to the RLU detection program. Three replicates of each concentration were processed and used for calculating the average.

Finally, the standard curve x- and y-axis were log-transformed using the base 10 for the ATP content (10^{-17} mol) and RLU; the graph function was y=ax+b. There was an individual standard curve for each detector.

2 Paired design

There were two principal methods, the colony counting method (C) and the ATP bioluminescence method; the latter was performed by three detector types (B, P, and N). In this study, each sampling was performed according to the two methods or detectors described above (paired design). Specifically, the two methods/detectors were used to sample different points on the same surface. That was, there were 6 groups of pairs, respectively C vs B, C vs P, C vs N, B vs P, B vs N, P vs N.

3 Sampling sites

Intensive care units (ICUs) and internal medicine wards were used as representative sites for type II and type III environments, respectively. The samples were collected from treatment vehicles, treatment tables, bedside cabinets, doorknobs, etc. ?

4 Sampling and test

The environmental surfaces were disinfected by chlorine with a disinfectant concentration of 500–1000 mg/L and sampled after the surfaces had dried. We sampled using the swabs in the ATP Surface Test or cotton swabs soaked in neutralizer (0.1% sodium thiosulfate). Surface samples in this study were collected by wiping a $100cm^2$ area with a sterilized specification plate (5cm×5cm). If the total surface at a site was smaller than $200cm^2$, each method or detector was used on half of the area.

The ATP bioluminescence method could be tested on site. The samples were shaken 20 times to mix the swab with the reagents after sampling and then analyzed by the detector to measure the RLU value. The results were reported as ATP content (mol) per surface site, using a standard curve for data conversion.

The colony counting method should be done in laboratory. After sampling, the nutritional agar culture medium (Qingdao Hope Bio-technology Co.,LTD., China) was used to monitor microbial contamination of each surface. After the sampling tube was fully shaken, inoculate 1.0 mL of the eluate with different dilution numbers to the plate, pour the molten nutrient agar medium cooled to 40 to 45℃ into 15 to 20 mL per dish, and culture in a 36±1℃ incubator for 48 hours, then count the number of colonies. The total colony count was transformed to CFU per surface site, which was calculated by multiplying the average colony numbers per dish with the sample dilution factor.