CSIRO PUBLISHING

Healthcare Infection, 2015, **20**, 108–114
http://dx.doi.org/10.1071/HI15011

Research

Time series evaluation of the 3M™ Clean-Trace™ ATP detection device to confirm swab effectiveness

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Abstract. ***Background*:** As of 2012, a downward trend in infection rates for hospital onset of both *Clostridium difficile* infections and methicillin-resistant *Staphylococcus aureus* bloodstream infections (2% and 4% decrease respectively) has been noted. Despite the success with these two organisms, several infectious pathogens in the healthcare setting have not decreased. This lack of downward trend highlights the importance of continuing to find and assess rapid detection methods to help confirm that hospital cleaning efforts meet and exceed standards of cleanliness demonstrated to reduce numbers of healthcare- associated infections (HAIs) of these pathogens.

***Methods*:** This study set out to determine the effectiveness of the swab 3M™ Clean-Trace™ Adenosine Triphosphate (ATP) System over time by comparing the ATP measurements of the culturable organisms to the corresponding quantitative microbiology. The organisms evaluated included: *Acinetobacter baumannii*, *Bacillus anthracis Sterne* endospores and vegetative cells, *Candida albicans*, *Clostridium difficile*, *Escherichia coli*, *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus* and *Mycobacterium smegmatis*.

***Results*:** A combined organisms analysis did not demonstrate a significant reduction in measured ATP levels over the course of the organisms' exposures in a controlled environment. The quantitative microbiology did, however, demonstrate a large initial organism die-off within the first 60 min ($P < 0.001$) of controlled environmental exposure, although the trend did not continue over the remaining 3 h of observation. The live versus dead experimental design yielded 100% microbial kill and a one log reduction ($P < 0.019$) between pre-exposure and post-exposure ATP measurements.

***Conclusions*:** This study did not demonstrate a significant effect of time in reducing ATP measures over the time periods evaluated. ATP measurements were approximately the same, regardless of the initial organism die-off. Additionally, the live versus dead analysis confirms that ATP bioluminescence is not sensitive enough to distinguish between viable organisms and organic debris remnants on sterilised equipment.

Additional keywords: adenosine triphosphate, ATP measurements, environmental persistence, healthcare-associated infections.

Received 13 May 2015, accepted 4 August 2015, published online 31 August 2015

Implications

- Concentrations of measureable adenosine triphosphate (ATP) on hospital-like surfaces do not decrease over the observed 4-h experimental time period.
- ATP bioluminescence cannot distinguish between viable and non-viable organisms.

Introduction

According to the Centers for Disease Control and Prevention (CDC) National and State Healthcare-Associated Infections Progress Report (2012), progress has been made in two major Healthcare-associated infections (HAIs).[1] The number of hospital-onset *Clostridium difficile* infections and methicillin-resistant *Staphylococcus aureus* bloodstream infections have decreased (2% and 4%) compared with national baseline average infection rates. The reported number of infections associated with these two organisms may be on the decrease; however, there are several pathogens of interest when discussing HAIs on the national level that have not demonstrated a downward turn in infection rates.[1]

Despite the decreases in the national average of *C. difficile* infections and methicillin-resistant *Staphylococcus aureus* bloodstream infections, much needs to be done to address and reduce infection rates of other organisms of concern in the hospital setting. The cleanliness of hospital and healthcare environments is a contributing factor to disease transmission in the healthcare setting.[2–5] There is a great need for a quick, reliable and easily interpreted assessment of surface cleanliness and detection of pathogenic microorganisms. Previous methodologies are either too time consuming (standard quantitative microbiology plating) or cannot confirm that the surface is adequately disinfected (fluorescence bio-marking). Adenosine triphosphate (ATP) bioluminescence has been used often in the food processing industry to determine cleanliness of surfaces in the food processing industry and its use has been well documented.[6] Using ATP detection methods employed in the food industry, researchers have begun to use ATP to determine cleanliness of hospital surfaces following decontamination.[7–10] The use of ATP bioluminescence in the healthcare setting has been driven by researchers looking to find a useful and reliable method for rapid detection of pathogen loads of common hospital surfaces.[7–10] Because of this great push towards ATP bioluminescence usage as a confirmation of surface cleanliness, there needs to be research to determine if ATP bioluminescence is capable of detecting changes in ATP levels due to microbial die-off and disinfection practices.

Previous work has attempted to determine if ATP bioluminescence is comparable to microbiological plate counts and therefore a potential rapid detection tool that may be used as a substitute to the 'gold standard' quantitative microbiological methods.[7–10] This research was limited and did not address if the ATP detection system was sensitive enough to detect decreases in ATP of environmentally persistent organisms left in the hospital or healthcare environment after cleaning occurs. Also, it did not assess whether or not there was a notable difference in the ATP measurements of viable and non-viable bacteria.

This study evaluated the correlation of ATP measurements (relative light units (RLU)/surface) and standard microbiological culturing methods (colony forming units (CFU)/surface) for nine organisms having a known association with HAIs or their surrogates. The goal was to determine if the ATP measurements demonstrated a decrease in measureable levels over various time points of the organisms being left for exposure to ambient air in a controlled environmental setting. The first study aim was to determine if the ATP bioluminescence test system was capable of detecting differences in microbial 'die-off' over a period of exposure to the air of the bio-safety cabinet (BSC). Additionally, we wanted to determine if the quantitative culture-based methods demonstrated a decrease in viable organisms over various evaluation time points. The second study aim was to determine the effectiveness of the swab 3M™ Clean-Trace™ ATP System at differentiating between viable and non-viable organisms. The goal was to determine if the ATP detection system was capable of distinguishing between viable and non-viable organisms by showing a decrease in ATP measurements between the 'live' organisms and the organisms 'killed' through steam sterilisation.

Methods

In order to determine the effectiveness of the 3M™ Clean-Trace™ ATP System (3M Health Care, St Paul, MN, USA) in detecting decreases in the number of viable organisms over an extended time, ATP measurements of various organisms were collected at pre-specified time points and compared with standard microbiological plate counts collected at the same time points. Additionally, we set out to determine the effectiveness of the 3M™ Clean-Trace™ ATP System to detect ATP in organisms rendered non-culturable through steam sterilisation. The following experiments were conducted utilising methodologies similar to our previous studies.[7,8]

For the time series experiment, organisms in phosphate-buffered saline suspensions were adjusted to the concentration of 2×10^7 CFU/mL. Five drops of 10 µL each were placed on triplicate (three for ATP and three for CFU evaluation) 100 cm^2 stainless steel surfaces (Steelworks, Cincinnati, OH, USA) bringing the surface concentration to ~10^6 CFU/cm^2. Surfaces were swabbed at time points of 0, 60, 120, 180, and 240 min and the swabs were analysed using the manufacturer's recommendation for the rapid 3M™ Clean-Trace™ ATP System test as well as standard culture methods.[7,8] Results were reported as either RLU/surface or CFU/surface.

For the second experiment – testing the ability of ATP to differentiate between viable and non-viable organisms – organism suspensions were prepared as before and 25 mL of each suspension was rendered non-viable through steam sterilisation at 121°C for 15 min.[7,8] The 3M Attest 1292 Rapid