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Evaluation of the relationship between ATP bioluminescence assay and the presence of organisms associated with healthcare-associated infections

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Abstract. ***Background*:** High prevalence and high mortality rates associated with healthcare-associated Infections (HAI) indicate there is a need to prevent HAIs from spreading. Cleaning and disinfection of hospital surfaces are fundamental to preventing HAIs, as is the confirmation of the success of these processes. Adenosine triphosphate bioluminescence has been identified as a quicker way to confirm cleaning, but questions remain regarding its specificity regarding microorganisms important to HAIs.

***Methods*:** This study evaluated ATP bioluminescence's efficacy in determining microbial contamination on 17 surfaces from the healthcare environment, and to determine if the ATP measurements of *Acinetobacter baumannii*, *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Mycobacterium smegmatis*, and methicillin-resistant *Staphylococcus aureus* corresponded to quantitative microbiology.

***Results*:** A strong positive correlation was discovered for each of the six organisms associated with HAIs, as well as an additional 'all organisms' analysis that combined all the six organisms.

***Conclusion*:** This study demonstrated a correlation between ATP bioluminescence measurements and quantitative microbiology; however, it was not as strong at low bacterial concentrations.

Additional keywords: *Acinetobacter baumannii*, Adenosine triphosphate (ATP), ATP measurement, *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, healthcare-associated infections, *Mycobacterium smegmatis*, *and* methicillin-resistant *Staphylococcus aureus.*

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Introduction

Healthcare-associated (nosocomial) infections (HAIs) are those infections caused by pathogens encountered in a hospital, hospital-like or other medical settings. In 2002 alone there were ~1.7 million nosocomial infections in US hospitals resulting in ~99 000 deaths.[1] High prevalence and high mortality rates associated with HAIs indicate there is a need to prevent the spread of these infections. Recent

Implications

- Our study demonstrates a correlation between ATP and six organisms important to HAI.
- Our study supports the use of ATP to confirm cleaning in the healthcare environment.

research has focused on cleaning and disinfection of hospital surfaces and fomites that have the potential to spread infection among hospital patients. In an effort to decrease HAIs, various methods of cleaning and decontamination are being utilised in the hospital setting.

The confirmation of cleaning and disinfection is important for both quality-control and quality-improvement efforts. However, current methods – such as visual inspection, fluorescent marking of surfaces or culture-based assessment, all have problems with cost, logistics or accuracy.[2,3] ATP bioluminescence has been identified as a quicker way to confirm cleaning, but questions still remain regarding its specificity regarding microorganisms that cause HAIs.[4–6]

Several studies have compared the 'gold standard' quantitative microbiology method with the ATP detection method in the healthcare environment and found variable correlation, usually attributed to the fact that quantitative microbiology measures only bacteria, while ATP measures all organic debris.[3,5,7,8] We felt that a comparison of the methods in the laboratory under controlled conditions – in order to minimise contamination – would provide useful information regarding the utility of ATP bioluminescence with specific microorganisms important to HAI.

The aim of this study was to evaluate ATP bioluminescence's efficacy in determining microbial contamination on surfaces commonly found in the healthcare environment, and to determine if the ATP measurements of several organisms known to be associated with HAIs corresponded to their concentration in the standard unit of measure (colony-forming units per surface).

Methods

Evaluations were conducted in the laboratory to determine if ATP bioluminescence results correlated with the standard culture-based methodologies for *Acinetobacter baumannii* BC9782, *Candida albicans* wild type, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Mycobacterium smegmatis* ATCC 14468, and methicillin-resistant *Staphylococcus aureus ATCC 43300* (MRSA). *Mycobacterium smegmatis* ATCC 14468 was used as it is a surrogate for *Mycobacterium tuberculosis*, while the remaining five microorganisms are pathogens associated with HAIs. Seventeen different surfaces, common to the healthcare environment, were used as coupons for this evaluation as were three concentrations of each microorganism of ~10^4, 10^6 and 10^8 colony-forming units per surface (CFU/surface). The actual coupons, either from surplus hospital equipment or purchased from a home improvement store, were either cut into 100 cm^2 or left their original size if close to the desired surface area. All coupons were cleaned and then sterilised before use. Results are given throughout the study as per total surface. Twelve coupons were used during each evaluation: three were negative ATP controls, three were negative culturable controls, three were ATP exposures and three were culturable exposures. Then each evaluation was conducted in triplicate for each of the surface concentrations (10^4, 10^6 and 10^8 CFU/surface). Five 10 µL drops of either an organism suspension in phosphate-buffered saline (PBS) to form the proper surface concentration for the exposure coupons or sterile PBS for the control coupons were used for each evaluation. After inoculation with organism suspension or PBS the coupons were left in a biosafety cabinet to dry for 10 min before they were swabbed.

In order to evaluate the culturable methods, each coupon was swabbed for 30 s with a moistened sterile polyester-tipped swab (Fisher Scientific, Waltham, MA, USA); then the swab tip was cut and placed into sterile PBS so that it could be vortexed for 30 s. The resulting organism suspension was then serial diluted in triplicate onto the appropriate media and incubated for a minimum of 48 h at 37°C with the colony-count results reported as CFU/surface. Positive and negative control measures were utilised throughout serial dilution and agar plating with any not within parameters resulting in the evaluation being discarded and repeated. The 3M™ Clean-Trace™ ATP Surface Test kit, including the 3M™ Clean-Trace™ ATP surface swabs and 3M™ Clean-Trace™ NGi Luminometer (3M Health Care, St Paul, MN, USA) was used to determine ATP levels. 3M™ Clean-Trace ATP surface swabs were used to swab the coupons for 30 s; then the swabs were activated and placed in the Clean-Trace NGi Luminometer. A quantitative digital readout gave the ATP measurement in the form of relative light units (RLU) with results reported as RLU/surface.

Organism suspension preparation

Depending on the nutritional needs of each microorganism, a pure culture of the appropriate microorganism was transferred from either an agar plate of tryptic soy agar without (MRSA) or with sheeps' blood (*A. baumannii*), brain heart infusion agar (*E. coli*, *E. faecalis*, *M. smegmatis*), or Sabouraud dextrose (*C. albicans* wild type) agar to a tube of 40 mL of tryptic soy broth, brain heart infusion broth, or Sabouraud dextrose broth. Multiple tubes of each organism were incubated at 37°C for a minimum of 24 h until sufficient growth was achieved.[9] A Beckman TH-4 rotor was used to centrifuge all tubes for 15 min at 3°C at 1000*g* with the supernatant decanted. This procedure was repeated at least twice with the resulting soft pellets combined and resuspended in PBS. The organism suspension was evaluated to confirm concentration.[9] The organism suspension was also evaluated before and after each evaluation for quality-assurance purposes with any