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Reliability Testing for Portable Adenosine Triphosphate Bioluminometers

Measurement of adenosine triphosphate (ATP) using portable bioluminometers has been adapted from the food manufacturing sector, and it has been suggested that it could be used as an indicator of surface soiling or cleanliness in hospital settings.¹ Some healthcare authorities are considering the use

of portable ATP bioluminometers as a tool for standard setting for surface cleanliness to improve cleaning standards.² Central to this approach is the use of a commonly accepted level of detected ATP—expressed as relative light units (RLUs)—that may be used as a surrogate for underlying soiling, including the presence of pathogenic microorganisms.³

It has been demonstrated that improvements can be made to cleaning processes with fluorescent markers through a simpler approach that provides a qualitative efficiency measurement of the cleaning process.⁴ Measurement of surface hygiene using ATP bioluminometers is thought to provide a more quantitative surrogate of surface cleanliness.⁵

A proposed ATP/RLU standard for acceptable cleanliness has been revised from 500 RLUs, originally suggested by Griffith et al,¹ to 250 RLUs by Lewis et al⁶ and most recently to 100 RLUs by Mulvey et al.⁷ However, a close examination of the references reveals that different brands of ATP bioluminometers were used to establish the data in each reference—a substantial problem given that each unit reads on a different relative scale.⁸ Even with a single branded unit variable results are observed without a clear explanation.⁹ There also is debate over the validity of using ATP measurement as an analogue for surface soils and the presence of pathogenic microorganisms.¹⁰

Our aim was to validate the reliability of measurement of 3 commonly available brands of portable ATP bioluminometers. Two of the brands selected (Hygiena and 3M) feature prominently in the published literature, and the third brand (Kikkoman) provided a different approach to luciferase presentation (a powder rather than a preprepared liquid).

Our method was selected to minimize confounding variables, such as brand-to-brand differences in RLU scaling, swab absorption, cell lysis mechanism and efficiency, liberation of cellular ATP, and variations in cellular ATP during bacterial cycles. To achieve this, the method used an ATP source of known purity (Sigma-Aldrich). The ATP was diluted across multiple dilution series, which enabled testing of the 3 devices across the full dynamic range of detection for each device, from the lower limit of detection to response tapering. We included multiple 10-fold dilution series as well as multiple narrower-range dilution series. A calibrated micropipette (Thermo Scientific) was used to apply the diluted ATP directly onto the swabs for each of the portable ATP bioluminometers, following an earlier method.¹¹

At each dilution point, each brand was tested in triplicate or more frequently. The swabs for each brand were from multiple batches, stored in accordance with the manufacturers' recommendations, and used within the use-by dates. High-performance liquid chromatography (HPLC; Shimadzu) was used to validate accuracy, precision, specificity, and linearity and as a quantitative control for ATP.

Materials used in our experiments included 667 ATP swabs in 153 separately measured dilution series (3M: 246 swabs in 57 runs; Kikkoman: 222 swabs in 49 runs; Hygiena: 199 swabs

in 47 runs). HPLC data were generated over 22 runs against 72 dilutions. ATP concentrations ranged from 10^{-5} mg/L to 1,000 mg/L.

Similar to many other studies, our results indicated considerable variability; data are shown in Figure 1. The raw data were subjected to test of association, which found no significant difference. The nonstandardized RLU scaling, which was different for each brand, required a single common calculation for comparison. The coefficient of variance was determined to be the best method of expression of the variability in RLU readings. The experimental method of multiple runs (153 test runs plotted in Figure 1) over the full dynamic range of detection did not allow for an overall standard deviation or mean to be determined for each device. HPLC demonstrated a lack of variability with excellent precision and repeatability of ATP analysis, provided that the limits of detection were observed.

The variability of the coefficient of variance shown in the ATP data for the 3 brands strongly suggests that these analytical systems were rarely able to produce a reliable response no matter the dilution or quantity of ATP. These findings have implications for interpretation of data obtained using a portable ATP bioluminometer when monitoring surface hygiene as part of infection control practice. Use of these devices in the food sector is based on a validation pathway quite distinct from the way in which usage has been trialed in the healthcare setting.^{1,3}

Portable ATP bioluminometer units are reliable for distinguishing between very different levels of ATP where the concentration is varied by at least an order of magnitude, thus reducing the relative impact of the variance.⁸ The usefulness of these units in healthcare settings is not ruled out by our

findings provided that usage allows for wide disparity in narrowly focused measurements of similarly clean or soiled surfaces. We caution against use of a finite measure of cleanliness based on RLUs until standard measures are improved and the units are able to read reliably on a common RLU scale, with a clear association with quantitated ATP levels.

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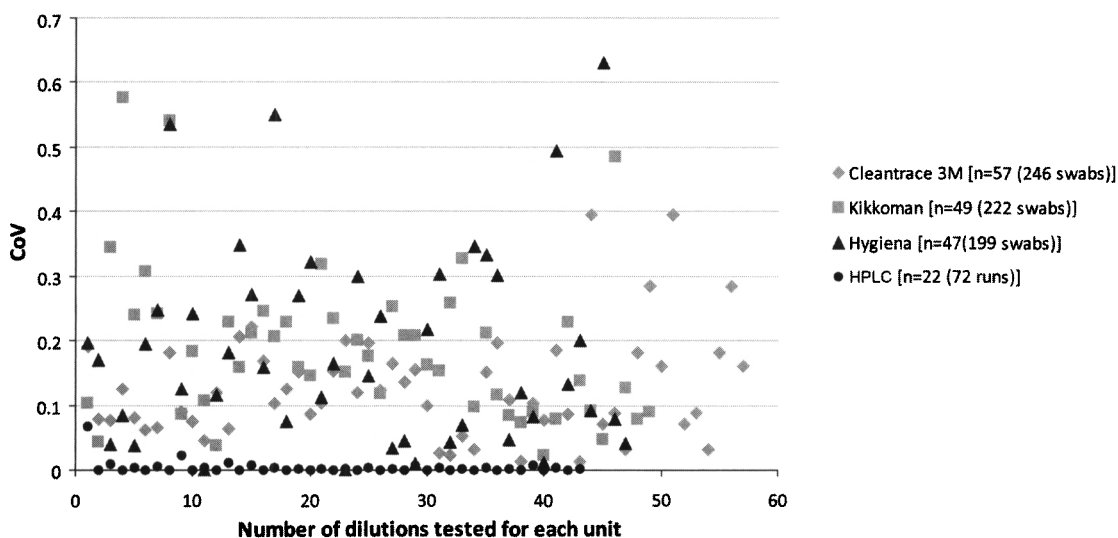


FIGURE 1. Coefficient of variance (CoV) for 3 portable adenosine triphosphate bioluminometers. *n* values indicate the number of separate dilutions tested per brand. HPLC, high-performance liquid chromatography.

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Is Environmental Contamination Associated with *Staphylococcus aureus* Clinical Infection in Maximum Security Prisons?

Over the past decade, large outbreaks of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections have occurred in correctional facilities across the country.^{1,2} Although many have been managed with aggressive interventions, response to standard infection control procedures has been variable, highlighting our incomplete understanding of staphylococcal transmission in this setting.² Environmental contamination has recently emerged as a possible target for novel prevention and control strategies.^{3,4} This study sought to characterize the relationship between environmental contamination and clinical infection in this vulnerable population.

We conducted a case-control study of *S. aureus* environ-

mental contamination at 2 New York State (NYS) maximum security prisons: Sing Sing (men) and Bedford Hills (women). Prisoners with documented *S. aureus* skin infections were identified by medical personnel at each prison. For every case, 2 uninfected controls—1 nasally and/or oropharyngeally colonized with *S. aureus* and 1 noncolonized—were selected from the same prison in a contemporaneous fashion. These were identified through our research group's ongoing study of *S. aureus* colonization in NYS prisons.⁵

Consenting study participants had a standardized set of environmental surfaces cultured within 1 week of infection diagnosis (cases) or selection (controls). These included bed sheets, sink handles, toilet flushes, toilet seats, cell bars, light switches, soap dishes, window handles, locker handles, and radios but varied on the basis of the prisoner's cell contents. Cultures were also obtained from shared gymnasiums in each prison at study initiation.

All samples were collected using premoistened rayon-tipped swabs and qualitatively cultured as described elsewhere.⁵ *S. aureus* isolates were typed by polymerase chain reaction sequencing of the *spa* (staphylococcal protein A) gene.⁶ SAS (ver. 9.2; SAS Institute) was utilized for data analysis. The study was approved by the Columbia University and NYS Department of Corrections Institutional Review Boards.

Ten cases were enrolled in this study. Twenty controls were selected, but 2 did not meet inclusion criteria. There were no significant associations between case status and the demographic and exposure variables assessed (sex, age, race/ethnicity, self-perceived health, shower frequency, and gym use). The proportion of subjects with *S. aureus* contamination on 1 or more surfaces did not vary appreciably on the basis of infection status (3/10 cases [30%] vs 6/18 controls overall [33.3%]; Table 1). Despite this, environmental contamination of controls varied depending on their colonization status. Surface contamination, when present, was more frequent among cases than among controls (13/18 surfaces from 3 cases [72.2%] vs 20/43 surfaces from 6 controls [46.5%]; $P = .07$). Six clonal types were identified on surfaces of the 9 contaminated cells; only 1 cell had more than 1 clone present. None of the infectious, colonization, or personal environmental isolates were methicillin resistant.

Of the 20 items sampled in the Sing Sing gymnasium, 8 (40%) were positive for *S. aureus*. These included the gym door handle, boxing gloves, basketballs, abdominal crunch machine, seated and upright leg presses, and hand sanitizer dispenser. Among these surfaces, 6 clonal types were found (*spa* t002, t008, t334, t701, t1510, and t2334), and all were methicillin susceptible. The Bedford Hills gymnasium was not heavily contaminated; 2 (7.7%) of 26 surfaces were positive, 1 with methicillin-resistant *spa* t008.

Few studies have assessed the prevalence and significance of bacterial surface contamination in jails or prisons. In 2009, Felkner et al⁷ cultured 132 surfaces from a Texas jail in a nonoutbreak setting. *S. aureus* was recovered from 10 surfaces (7.6%), with the majority of isolates (8/10) resistant to meth-