Comparison between adenosine triphosphate bioluminescence and aerobic colony count to assess surface sanitation in the hospital environment

Daniele Domenico Raia[1], Lucia Cannova[1], Sandro Provenzano[1], Omar Enzo Santangelo[1], Dario Piazza[2], Enrico Alagna[1], Valentina Bonanno[1], Luigi Aprea[3], Alberto Firenze[1,3]

(1) Department of Sciences for Health Promotion and Mother–Child Care "G. D'Alessandro", University of Palermo, Italy
(2) Regional Reference Center for the Care and Diagnosis of Rare and Hereditary Tumors – Oncology Unit, Palermo University Hospital "Policlinico P. Giaccone"

CORRESPONDING AUTHOR: *Daniele Domenico Raia, danny2003@libero.it danieledomenico.raia@unipa.it, Via del Vespro, 129 - 90127 PALERMO (PA) - +3909123869306 - +3909123867510*

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ABSTRACT

Background: Adenosine triphosphate bioluminescence produced by the firefly luciferase has been successfully introduced to verify cleaning procedures in the food industry according to the Hazard Analysis Critical Control Point program.
Our aim was to evaluate the reliability of bioluminescence as a tool to monitor the effectiveness of sanitation in health-care settings, in comparison with the microbiological gold standard.
Methods: 614 surfaces of various material were randomly sampled in Policlinico University Hospital units in Palermo, Italy, to detect adenosine triphosphate bioluminescence and aerobic colony count. Linear regression model and Pearson correlation coefficient were used to estimate the relationship between the two variables of the study.
Results: Aerobic colony count median was 1.71 colony forming units/cm^2 (interquartile range = 3.8), whereas adenosine triphosphate median was 59.9 relative light units/cm^2 (interquartile range = 128.3). Pearson coefficient R^2 was 0.09. Sensitivity and specificity of bioluminescence test with respect to microbiology were 46% and 71%, whereas positive predictive value and negative predictive value were 53% and 65%, respectively.
Conclusion: According to our results, there seemed to be no linear correlation between aerobic colony count and adenosine triphosphate values, suggesting that current bioluminescence technology has not any proportional relationships with culturable microbes contaminating environmental surfaces in health-care settings.

Key words: Bioluminescence; Sanitation; Surfaces; Cleaning Monitoring.

BACKGROUND

Nosocomial Hospitalenvironmentsurfaces provide a reservoir of for microorganisms, including several pathogens, whichwhich represent apotential risk of nosocomial infections can survive for months onto inanimate surfaces [1]; therefore, thorough cleaning plays a pivotal role in order to reduce the hazard of health-care associated infectionssuch pathogenscan survive for months onto inanimate surfaces, so that thoroughcleaningis pivotal to reduce the hazard of transmission [2]. Nevertheless, it is not clearunanimous which strategies are optimal to rapidly monitor cleaning efficacy during everyday hospital activity [3]. Microbiological assays are have been considered regarded as as the gold standard in current cleanliness verification protocols, but they are expensive and time-consuming [4]. Bioluminescence has been proposed as a substituteintroduced to detect surface microbial contamination since relying upon the ubiquitously presence of adenosine triphosphate (ATP) is ubiquitously presentin microorganisms the cells of microorganisms [5]; this technology has become well established in the food processing industry as part of general Hazard Analysis and Critical Control Points (HACCP) measuresprogram, thanks to its ability to provide results within minutes [6,7].

The aim of the present study was to compare ATP bioluminescence and aerobic colony count (ACC) in order to assess whether a linear correlation exist between them.

METHODS

A total of 614 surfaces among those in closest contact to patients and healthcare workers have been analyzed in a twelve-months period. The composition material included stainless steel (n=211, 34.4%), engineering plastics (n=143, 23.3%), glass (n=67, 10.9%), neoprene (n=52, 8.5%), epoxy varnished steel (n=46, 7.5%), acrylonitrile-butadiene-stirene (n=28, 4.6%), epoxy varnished melamine wood (n=15, 2.4%), chromed steel

(n=14, 2.3%), cotton (n=14, 2.3%), marble (n=11, 1.8%), epoxy varnished mortar (n=10, 1.6%), polyvinyl chloride (n=3, 0.5%).

Slides pre-coated by the manufacturer (OXOID®, Cambridge, UK) with plate count agar (55 mm in diameter, 24 cm^2 wide) were pressed for 15 seconds onto each surface to be sampled and then incubated aerobically at 37°C for 48 hours. Visual assessment according to manufacturer's instructions was used to determine ACC, expressed in Colony Forming Units/cm^2 (CFU/cm^2).

A close zig-zag pattern swab in an adjacent 100 cm^2 area of the same surface was then assessed by means of Lumicontrol II® (PBI International, Milan, Italy) to measure ATP levels according to manufacturer's recommendations, and measures were expressed in Relative Light Units (RLU).

Hygienic failure thresholds We were set to chose 100 RLU/cm^2 and 2.5 CFU/cm^2 as hygienic failure thresholds for ATP bioluminescence and ACC, respectively, according to previous studies [8].

Data were collected and organized in a Microsoft Excel® database and then analyzed using by means of commercially available statistic software package StataSE-64-®.

ACC and ATP measures results were summarized as median (interquartile range, IQR).

Sensitivity of bioluminescence with respect to ACC gold standard was calculated as the ratio between the number of surfaces exceeding ATP benchmarks (<100 RLU/cm^2) among those exceeding also microbiological threshold (<2.5 CFU/cm^2, n=117) and the total amount of samples exceeding the ACC threshold (n=241).

ATP assay specificity was defined as the ratio between the number of surfaces whose values were below bioluminescence benchmark among samples with less than 2.5 CFU/cm^2 (n=255) divided by the overall quantity of surfaces below the ACC threshold (n=359).

The ratio between contaminated surfaces according to bioluminescence which exceeded also the threshold of microbiological test (n=117) and the total amount of positive ATP samples (n=221) yielded the positive predictive value of bioluminescence.

Negative predictive value was computed as the ratio between negative ATP samples among surfaces and the total count of negative ATP results (n=393).

Pearson correlation coefficient and linear regression model were used to describe the relationship between logarithmic transformed ATP values and ACC. Statistical significance was set as $p \leq 0.05$, two-tailed.

RESULTS

Overall, ACC had median values resulted inof 1.71 CFU/cm^2 (IQR = 3.8), whereas ATP surfaces median was 59,9 RLU/cm^2 (IQR = 128.3).

ACC benchmark was exceeded by 255 surfaces (41.5%); on the contrary,221 surfaces (36.0%) exceeded the ATP threshold; both benchmarks were exceeded simultaneously by 117 samples (19.0%).

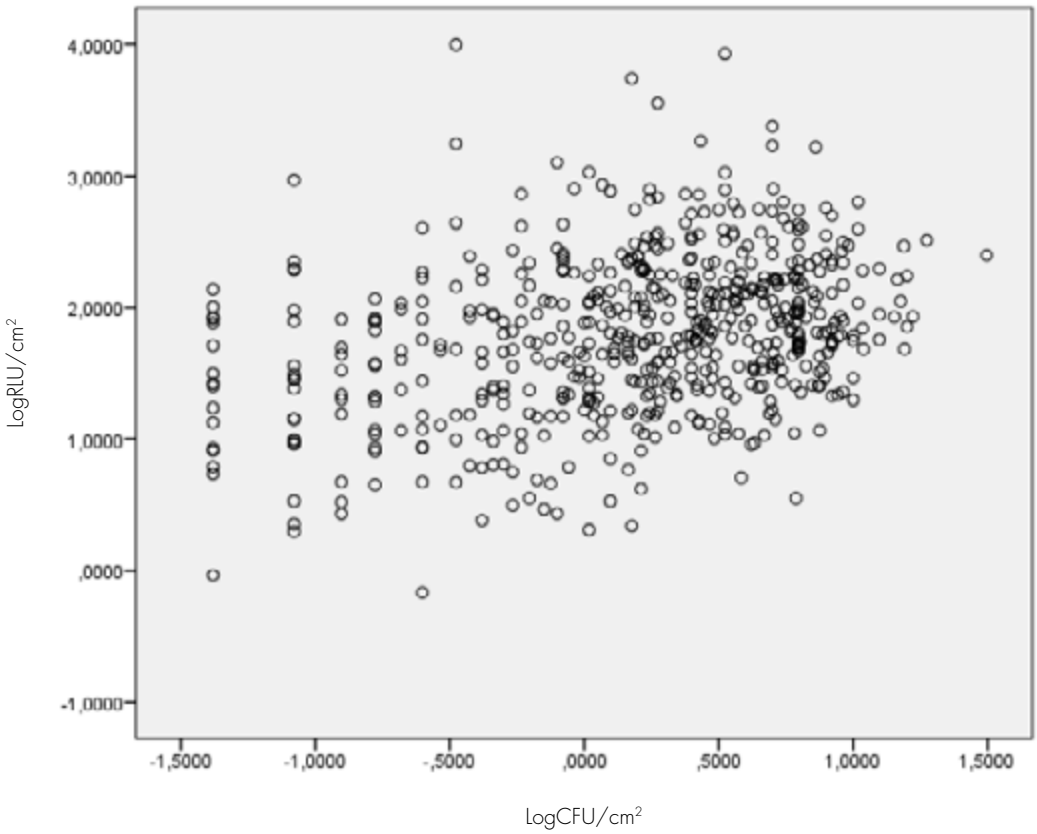
Fig. 1 shows the scatter plot between logarithmic transformed ATP and ACC, with Pearson coefficient R^2 = 0.09 (F-statistic = 64.6; p-value < 0.001).

Sensitivity and specificity of bioluminescence test with respect to ACC were 46% and 71%, whereas positive predictive value and negative predictive value were 53% and 65% respectively.

CONCLUSION

The main strenght of the present study stands on the large number of sampled surfaces, larger than previous

FIGURE 1. Linear regression graph between logarithmic transformed ATP and ACC; Pearson coefficient R2 = 0.09 (F-statistic = 64.6; p-value < 0.001).



studies, set in hospital environment, found in literature.

In our research, Pearson coefficient R^2 (0.09) showed there was no linear correlation between ACC count and ATP values. One explanation could be based uponThis could be explained considering the fact that ACC measures the number of cultivable viable organisms, whereas bBioluminescence reveals all residual organic debris, so that minute traces of blood, urine or food could significantly increase ATP readings, yet have little effect on viable surface counts [9].

Venkateswarana et al. proposed a bioluminescence method that differentiated intracellular ATP (viable microbes) from free extracellular ATP (lysed, dead cells, etc.) by means of an ATPase which selectively hydrolized the latter [10]. This distinction proved to be of great importance since the levels of free extracellular ATP were extremely high, sometimes 2 or 3 orders of magnitude greater than the intracellular values.

Moreover, microbes have varying levels of ATP, according to the specific strain and metabolic activity, so that ATP content can not be correlated statistically with CFU count, nor the amount of measured ATP can be used to quantitatively estimate bioburden quantitatively, due to large differences in volumes and metabolic states of different cells [6,7].

Non-culturable species in the media provided could not give their contribution to CFU count and this represent an important limit of the study, resulting in underestimation of certain viable microbial population [11]. Secondly, swabs were not taken from the same areas but from adjacent ones, in order to avoid further contamination, but we can not exclude different readings.

In summary, despite differentiating between intracellular versus extracellular ATP, ATP levels did not correlate linearly with the plate counts.

Bioluminescence assay was proposed to rapidly assess environmental contamination during routine hospital activities; therefore sampling was performed independently from the schedule of the hospital cleaning service company. Moreover, differences in the materials composing the sampled surfaces might affect microbiological contamination and ATP results [12]. The lack in homogeneity of sampled surfaces and in standardized cleaning conditions can be considered as one major limit of the present study.

The use of bioluminescence alone does not seem to be sufficient in order to predict nosocomial infection hazard. Integration with mMicrobiological techniques, supplemented with DNA analysis, could be the best compromisestill represent the gold standard to assess surface contamination with hygienically relevant microorganisms, since healthcare associated infection risk is more likely to be related to surface contamination withspecific

pathogens, and sampling for these is more relevant when circumstances indicate suggest their presence, e.g. during outbreak investigations or after a colonised patient has inhabited a room [12].

Since comprehensive cleaning remains a milestone for disrupting the chain of infection between pathogens and patients, the research for a reliable, cost- and time-effective method to assess its accuracy in everyday hospital practice still represents an important issue.

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Conflict of interest

All authors deny any conflict of interest.

Financial disclosure

None of the authors had any financial support for the study.

Authors' contributions

LA, AF and LC conceived, designed, coordinated and supervised the research project. DDR, SP, OES, EA and VB collected samples. DDR, SP, OES and DP performed the data quality control, optimized the informatics database, performed the statistical analyses and evaluated the results. DDR wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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