

**Evaluation of ATP
bioluminescence swabbing as a
monitoring and training tool for
effective hospital cleaning**

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Acknowledgements

We are grateful to NHS Estates for funding this study, and to Hygiena International Limited for the supply of ATP bioluminescence swabs and a luminometer. We also thank the staff of the wards involved in this study for their help and co-operation.

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Abstract

It is well documented that effective cleaning in hospitals is an important aspect of infection control of organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter* species. There is a need for simple, rapid methods of assessing cleanliness in order to effectively audit cleaning programmes and to educate staff. The aim of this study was to evaluate the use of ATP bioluminescence swabs for assessing cleanliness in hospitals. Sites ($n = 108$) in three hospital wards, including floors, patient equipment and clinical workstations, were examined by visual assessment, microbiological swabbing and ATP bioluminescence swabbing. Overall, ATP bioluminescence swabbing detected a slightly larger number of contamination problems than the other methods, with a particularly large proportion of workstations failing by this method compared to visual or microbiological assessment. Highest mean contamination levels (both ATP and microbiological) were obtained from floor sites under patient beds, and the lowest levels from patient equipment. ATP bioluminescence swabbing was used during education sessions with ward staff and cleaning staff, to demonstrate the importance of cleaning, with an immediate anecdotal increase in enthusiasm for cleaning. Long-term changes in cleaning behaviour following education proved more difficult to evaluate.

1 Introduction

Healthcare-acquired infections (HCAI) cause considerable complications to patient recovery, with patients who acquire an infection during in-patient stays remaining in hospital for an average of 14 extra days and having a seven-fold increase in risk of death (1). It has been estimated that 321,000 patients per year acquire infections whilst staying in hospital in England, with an estimated cost of almost £1 billion per year (2). The issue of hospital cleanliness is therefore currently high on the political agenda in the United Kingdom. Several initiatives have been introduced by the Government in recent years to address this problem, such as the Secretary of State's 'Towards cleaner hospitals and lower rates of infection' and the Matron's Charter, both introduced in 2004 (3). It is well documented that effective cleaning in hospitals is an important aspect of infection control of organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) (4), vancomycin-resistant enterococci (VRE) (5), norovirus (6) and *Acinetobacter* species (7).

It has been demonstrated that visual assessment alone is a poor indicator of cleaning efficacy (8,9). Microbiological monitoring, by means of surface swabbing followed by bacterial culture in the laboratory, is one method by which cleaning efficacy can be evaluated. However, since the results of bacterial culture may not be available for several days, it is difficult to use this method for rapid demonstration of cleaning problems to healthcare and domestic staff. An alternative method of monitoring environmental contamination, which has been used by the food industry for several years, is the detection of adenosine triphosphate (ATP) by a bioluminescence reaction (10,11). ATP is present in all living organisms, including microorganisms, and its presence therefore acts as an indicator of both microbial contamination and contamination with food, drink or bodily fluids that may support microbial growth (12). Thus, a large quantity of ATP on a surface after cleaning and disinfection is an indication of poor cleaning, and therefore a contamination risk. This technique involves surface swabbing, followed by reaction of any ATP with luciferin and luciferase enzyme, resulting in the emission of light (one photon of light is emitted for each molecule of ATP present). The light is detected using a hand-held

luminometer, giving a result in a few seconds. The rapid result is a significant advantage over conventional microbiological swabbing. In addition, the ease of use of this technique, and the portable nature of the equipment, are advantageous compared to microbiological swabs that must be processed by skilled staff within a laboratory.

The rapid availability of ATP results provides the potential to educate cleaning staff in real time, and to implement immediate corrective action if cleaning is proved to be inadequate. In the food industry, this method is commonly used to check that surfaces have been cleaned to the required level, and surfaces giving an unsatisfactory ATP result can be re-cleaned before food production begins (12). Whilst the technology has been well validated in the food industry, there is little data regarding the use of ATP bioluminescence in the healthcare setting.

The aim of this study was to evaluate the use of ATP bioluminescence swabs for assessing cleanliness in hospitals, and to determine whether this method might be used to educate healthcare and cleaning staff and supervisors regarding appropriate cleaning techniques.

2 Materials and methods

Sampling sites

Over a period of three months, 108 sites in three hospital wards were examined by visual assessment, microbiological swabbing and ATP bioluminescence swabbing. These included 54 floor areas under patient beds, 17 commode seats, 19 pieces of patient equipment (for example sphygmomanometers and Volumed pumps) and 18 clinical workstations.

Visual inspection

The amount of dust on each surface was determined by wearing a white vinyl glove and running a finger across the surface. A record was kept of the quantity of dust (0 = no dust; 1 = few specks of dust; 2 = visible layer of dust; 3 = heavy film of dust) and colour (white, pale grey, dark grey, black). Details of any other types of dirt or debris observed were also recorded.

Microbiological swabs

SpongeSicle swabs (Biotrace International, Bridgend, UK), impregnated with neutralising buffer, were used to swab an area of 100 cm², which was marked out using a sterile template (Technical Service Consultants Ltd, Heywood, UK).

ATP bioluminescence swabs

Hygiena Ultrasnap swabs (Hygiena International Ltd, Watford, UK) were used to swab an area of 10 cm², which was demarcated using a sterile template. ATP results were read using a systemSURE II ATP luminometer (Hygiena International Ltd).

Microbiological analyses

Enumeration of indicator organisms

A 10 ml quantity of Maximum Recovery Diluent (MRD) was added to each SpongeSicle swab in a sterile stomacher bag, and the swab was placed in a stomacher (AES Laboratoire, Combourg, France) for 60 seconds. The MRD suspension was then used to inoculate media for the enumeration of Aerobic Colony Count,

Enterobacteriaceae and *Staphylococcus aureus* according to HPA Standard Operating Procedures (13–15). In addition, enterococci were enumerated by inoculation of 0.5 ml aliquots of MRD suspension onto Slanetz and Bartley agar (Oxoid Ltd, Basingstoke, UK); plates were incubated at 37°C for 48 hours.

Identification of MRSA

Following enumeration and confirmation of *Staphylococcus aureus* by the procedure described above, a maximum of five confirmed colonies from each swab were sub-cultured onto Mannitol Salt Agar (E&O Laboratories, Bonnybridge, UK) with added oxacillin (8 mg/L), and plates were incubated at 37°C for 24 hours. Growth on this agar indicated resistance to oxacillin, and these organisms were therefore considered to be presumptive MRSA.

Identification of VRE

Following enumeration and confirmation of enterococci by the procedure described above, a maximum of five confirmed colonies from each swab were sub-cultured onto Slanetz and Bartley agar containing vancomycin (4 mg/L); plates were incubated at 37°C for 48 hours. Growth on this medium presumptively identified the organisms as VRE.

Interpretation of results

For visual inspection, a surface was considered to be satisfactorily clean if no dust or debris was observed, or if only a few specks of white dust were present. A small amount of dust (recorded as “1”) of a pale grey colour was considered to represent intermediate results. A quantity of dust recorded as 2 or 3, or a dark grey or black colour, was considered to represent a failure of cleaning (it is generally considered that dust becomes darker in colour over time, thus a darker colour equates to older dust).

Aerobic Colony Counts were considered unsatisfactory if they were above 1.0×10^3 colony-forming units (cfu) per swab, as indicated in the guideline document produced by Campden and Chorleywood Food Research Association

(CCFRA) (12). Presence of Enterobacteriaceae, enterococci (including VRE) or *Staphylococcus aureus* (including MRSA) was considered unsatisfactory.

An ATP level of <100 Relative Light Units (RLU) was interpreted as satisfactory, 100–300 as intermediate and >300 as unsatisfactory.

Education of staff

Healthcare and cleaning staff from one ward were educated about the importance of hospital cleaning by means of informal teaching sessions given by a microbiologist and an Infection Control Nurse.

Staff involved in the session each took part in ATP bioluminescence swabbing of various surfaces in the ward environment, to demonstrate differences in cleanliness of these surfaces.

Monitoring of cleanliness following education sessions

Following the education sessions, cleanliness in the ward was monitored by means of regular audits (carried out by the ward sister together with a supervisor from the cleaning contractor), as well as ATP bioluminescence monitoring, for a period of five weeks.

3 Results

Comparison of ATP bioluminescence results with microbiological analyses and visual assessment

As Table 1 shows, the ranges of results for both microbiological and ATP swabs were wide, with highest results (both ATP and microbiological) being obtained from underneath beds, whilst lowest results by both methods were from patient equipment.

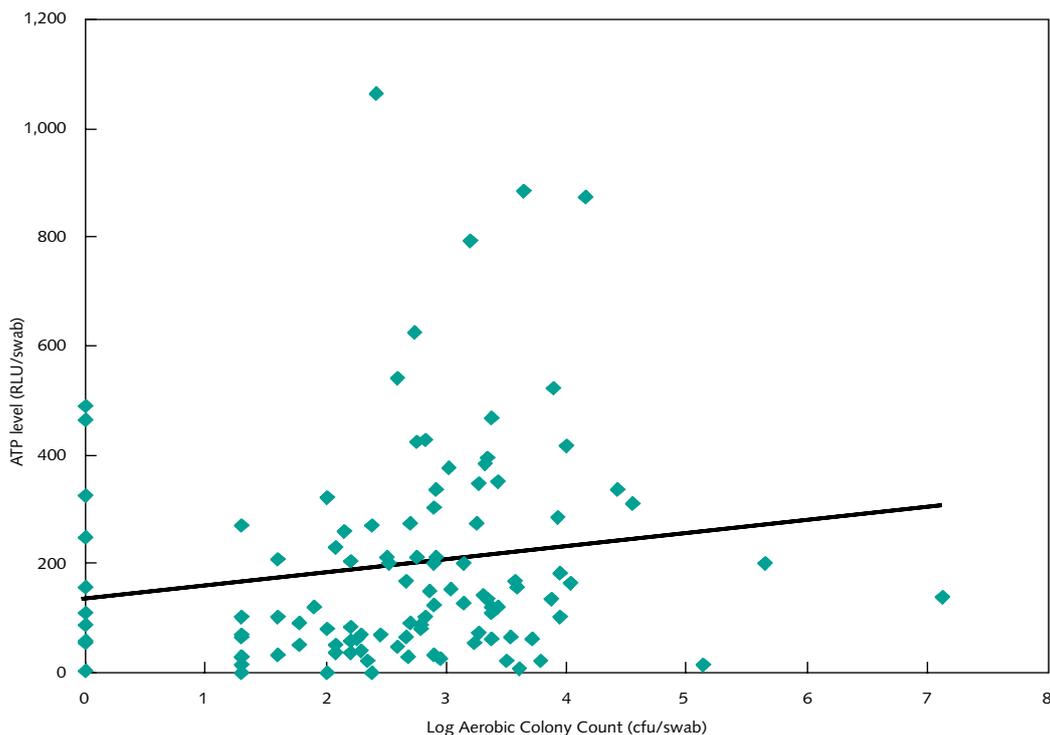
If ATP values and Aerobic Colony Counts are compared directly, there is poor correlation between the two methods (Figure 1). However, if the proportions of samples that pass or fail for each method are compared, there is a better relationship between results obtained by the two methods (Figure 2).

Table 1 Results of ATP bioluminescence swabs and microbiological swabs (Aerobic Colony Count) at different hospital sites

	Mean ATP level (and range) (RLU/swab)	Mean Log ₁₀ Aerobic Colony Count (and range) per swab
Under beds	269 (14–1,065)	2.94 (0–7.12)
Commode seats	127 (0–429)	2.55 (0–5.64)
Patient equipment	70 (1–207)	2.07 (0–4.03)
Nurses' workstations	164 (32–873)	2.20 (0–4.16)

Of all the sites sampled, 18% were deemed to be unsatisfactory by visual inspection for cleanliness, and a further 26% were of an intermediate quality (Figure 2); in comparison, 45% of sites gave unsatisfactory microbiology results, whilst the ATP method gave 22%

Figure 1 Correlation between ATP bioluminescence result (expressed in RLU/swab) and Aerobic Colony Count (cfu/swab)



unsatisfactory and 37% intermediate results. Thus, the overall proportion of satisfactory results by visual assessment was significantly greater than by ATP swabbing (chi-squared test, $p = 0.02$), and bacteriological swabbing also produced a higher number of satisfactory results than the ATP technique, with a borderline level of significance (chi-squared test, $p = 0.048$). There was no significant difference between visual assessment and microbiological swabbing.

Results for individual sites indicated variations in correlation between the three methods in different situations. For example, visual assessment of the floor under patients' beds detected more contamination problems than microbiological swabbing, and almost as many as ATP swabbing (Figure 2). However, for commodes, other patient equipment and workstations, visual assessment was considerably less sensitive than swabbing techniques. ATP swabbing was considerably more sensitive than other methods for detecting contamination on workstation surfaces.

Detection of pathogenic bacteria

Staphylococcus aureus was isolated from ten sites, including seven sites underneath beds, two commode seats and one workstation, with bacterial numbers ranging from 20 to 3.2×10^6 cfu per swab. Of these,

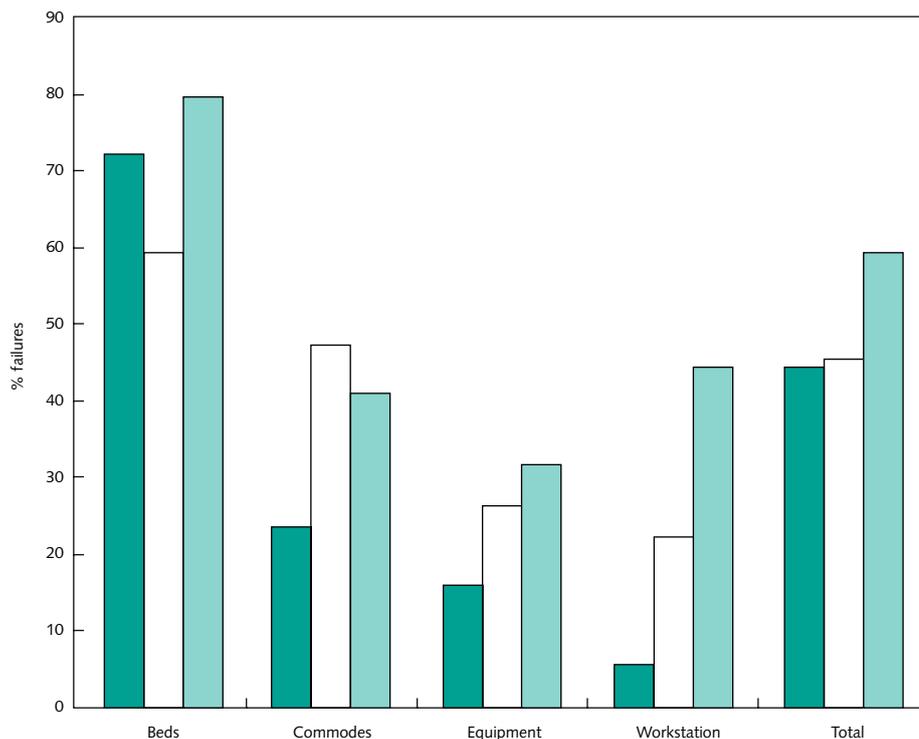
six isolates were identified as MRSA: five from a surgical ward (one commode seat and underneath four patient beds), and one from underneath a bed in the Intensive Care Unit. Whilst four of these sites failed visual assessment, two were considered acceptably clean by visual analysis. Moreover, five of these sites gave unsatisfactory levels of ATP but one ATP swab gave a low (satisfactory) result.

Enterococci were detected in 22 swabs (16 from underneath beds, three from equipment, two from commode seats and one from a workstation), with counts ranging from 20 to 2.6×10^4 cfu per swab. Only two of these isolates were presumptively identified as VRE. One was from underneath a bed in a surgical ward; this gave an unsatisfactory result from visual inspection and an intermediate ATP bioluminescence result. The other was from a piece of patient equipment in the oncology unit; both visual inspection and ATP swabbing at this site gave satisfactory results.

Education of staff regarding hospital cleanliness

Feedback immediately after teaching sessions was positive, with one group of staff carrying out a "deep clean" of the ward area immediately after the session. ATP bioluminescence swabs were seen as a novel way of

Figure 2 Percentage of sampling sites giving "failures" (ie intermediate or unsatisfactory results) by visual inspection (■), microbiological swabbing (□) or ATP bioluminescence testing (▒)



illustrating the need for cleaning, and therefore generated considerable interest and enthusiasm.

Long-term monitoring of standards of cleanliness after education sessions proved more difficult to achieve. Auditing was not carried out as regularly as had been hoped by the ward sister and cleaning supervisor, and ATP bioluminescence results alone did not give an appropriate overall impression of ward cleanliness. Table 2 illustrates the data that was available after a five-week period. It is clear that there were still areas where cleaning standards were not satisfactory, but the data is not adequate to determine whether an overall improvement in cleaning was achieved after the education sessions.

Table 2 Monitoring of cleanliness in ward area following staff education sessions

		Week 1	Week 3	Week 5
ATP results	Under bed	Satisfactory	Satisfactory	Intermediate/ Unsatisfactory
	Commode	Satisfactory	Satisfactory	Satisfactory
	Equipment	Satisfactory	Satisfactory	Intermediate
	Workstation	Unsatisfactory	Satisfactory	Unsatisfactory
Audit results	Patient area	Intermediate	N/A	Intermediate
	Clean store	Unsatisfactory		Unsatisfactory
	Bathrooms	Intermediate		Satisfactory
	Dirty utility	Satisfactory		Satisfactory

N/A: Information not available

4 Discussion

Improving and maintaining hospital cleanliness is an important aspect of the drive to reduce rates of HCAI. In order to achieve this, there is a need for simple, rapid methods of assessing cleanliness in order to effectively audit cleaning programmes and to educate staff. Results from this study, in agreement with previous studies (8,9), indicate that visual assessment alone is not an adequate indicator of microbial contamination. Although visual inspection was a fairly effective way to detect cleanliness problems on the floor under patient beds, this technique resulted in a significant under-estimate of contamination problems in other sites.

ATP bioluminescence swabbing has been used effectively to monitor cleanliness in food production premises for some years (10,11). Moreover, one study found it to be an effective method of monitoring hygiene in dental surgeries (16). Results from this study indicate that this technique may also be a useful indicator of overall contamination levels in hospitals. It can be seen from [Figure 2](#) that ATP bioluminescence results correlated better with the other techniques in some sites than in others. For example, on patient equipment and commodes, microbiological and ATP swabbing gave similar results whilst visual inspection under-estimated the number of failures. In contrast, visual inspection detected contamination under patient beds more frequently than was detected by microbiological swabbing. On floor areas such as these, contamination is largely in the form of dust that is visible to the naked eye, whereas equipment and commodes are rarely dusty, but may be contaminated with a small quantity of body fluids or other source of microbes that cannot be detected visually. ATP swabbing was particularly sensitive to contamination of clinical workstations compared to the other methods. Since these are surfaces that have a lot of hand contact, and possibly contact with organic material such as flowers, food and drink, this may explain why the ATP level was often high despite the relatively low rate of microbiological contamination.

Whilst it is clear that ATP swabbing is not directly equivalent to microbiological monitoring in terms of detecting contamination, it does seem to give a good indication of whether or not a surface has been well

cleaned. However, there were individual exceptions to this general correlation which might be a cause for concern if ATP were to be used alone as a marker for cleanliness. For example, on one occasion MRSA was detected under a bed whilst the ATP result was satisfactory. Similarly, VRE was detected on a sphygmomanometer cuff from which a satisfactory ATP result was obtained. Therefore, it may be more appropriate to use ATP bioluminescence swabbing in combination with other audit tools rather than relying on this method alone. For example, following an evaluation of hospital cleaning regimes, Griffith et al (8) recommended an integrated monitoring programme that used ATP bioluminescence in conjunction with visual and microbiological assessments. Thus, a visual inspection would be carried out initially. If this gave unsatisfactory results, the surface would be re-cleaned. If the surface were visually clean, but classified as high risk, ATP swabbing would be carried out and remedial action implemented where necessary.

In determining the correlation between microbiological and ATP bioluminescence results, it is important to set the “satisfactory/unsatisfactory” cut-off level for each method appropriately. There is no single recognised standard for interpretation of results of environmental swabbing, and levels considered to be acceptable vary markedly in different publications. Dancer (17) proposed that there should be $<1/\text{cm}^2$ of indicator organisms such as *Staphylococcus aureus* (including MRSA), VRE, *Clostridium difficile* and *Salmonella* in the clinical environment, and that frequent hand touch surfaces in hospitals should have an Aerobic Colony Count of $<5 \text{ cfu}/\text{cm}^2$ (equivalent to $<5.0 \times 10^2 \text{ cfu}/\text{swab}$ in our study). Meanwhile, Griffith et al (8) used $2.5 \text{ cfu}/\text{cm}^2$ (equivalent to $2.5 \times 10^2 \text{ cfu}/\text{swab}$) as the upper limit of acceptability for bacterial counts, and 500 RLU for ATP bioluminescence. These studies both have lower threshold levels for bacterial counts than that used in our study ($1.0 \times 10^3 \text{ cfu}/\text{swab}$). However, both studies focused largely on frequent hand touch surfaces whereas the evaluation we describe included floors as well as hand touch surfaces. Studies carried out by Campden and Chorleywood Food Research Association suggested that

it is possible to achieve at least a three log reduction in microorganisms attached to a surface following suitable sanitation procedures, and therefore a heavily contaminated surface (with an initial bacterial count exceeding 10^6 cfu/swab) should have the bacterial count reduced to 1.0×10^3 cfu/swab after cleaning (12). Both hospital floors and hand contact surfaces may be heavily contaminated immediately before cleaning, and therefore the cut-off level of 1.0×10^3 cfu/swab seems appropriate for our study. Given that 59% of floors swabbed, 47% of commodes, 26% of equipment and 22% of workstations were considered unsatisfactory, this criterion also appears reasonable in terms of achievability.

An ATP level of <100 RLU/swab was suggested as satisfactory (12), 100–300 as intermediate and >300 as unsatisfactory. Whilst these are the same RLU levels used in our study, it should be noted that these units of measurement are “relative” and are not necessarily comparable from one brand of luminometer and ATP swab to another.

Another factor which may affect the correlation of ATP bioluminescence and microbiology results is the presence of sanitisers on the surface of interest. It has been demonstrated that commercial sanitisers and cleansers such as quaternary ammonium, iodine cleaner-disinfectant, acid sanitiser and chlorinated alkaline cleaner may cause increases or decreases in the ATP bioluminescence result if the chemical comes into direct contact with the ATP bioluminescence reagents (18). Therefore, further investigation into the specific effects of sanitisers used in the hospital environment would be recommended before using ATP swabbing to monitor hospital cleanliness.

It is important that both cleaning and healthcare staff take responsibility for the condition of the hospital environment, and the Matron’s Charter, published in the UK in 2004, specifically states that “keeping the National

Health Service clean is everybody’s responsibility” (19). On one occasion during our study, a high count of *Staphylococcus aureus* was isolated from the floor despite the area having been mopped immediately before sampling. Although the risk of transmission from floors is low compared to hand contact surfaces, it is reasonable to expect that the bacterial load would be low immediately after effective cleaning. Therefore, this example highlights the need for education regarding the importance of thorough cleaning.

The use of ATP swabs during education sessions, to illustrate the need for cleaning, generated renewed interest in ward cleanliness amongst the staff involved. This was partly because it was something of a novelty to take part in a practical activity, rather than simply having to sit and listen to a talk on infection control. Furthermore, the generation of quantitative data from each swab provided a much clearer comparison of clean and dirty areas, compared with subjective visual examination. The immediate “deep cleaning” of the ward by staff after one education session was a particularly positive response, but it is also desirable that a more long-term improvement in cleaning is achieved by education sessions such as these. This long-term effect proved difficult to monitor in our study, and more attention would need to be given to the type and frequency of auditing tools to be used in future studies of this kind.

This study has demonstrated that ATP bioluminescence swabbing is a useful indicator of cleanliness in the hospital environment, and has given an indication that this technique may be useful as an educational tool. In the future, it would be useful to carry out a longer-term study to determine how frequently ATP swabbing should be used to verify cleaning procedures and how frequently staff need to be re-trained in order to sustain an improvement in ward cleanliness.

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