NHS National Services Scotland

Literature review and practice recommendations: Existing and emerging technologies used for monitoring the effectiveness of decontamination of the health and care environment

ATP Bioluminescence and Fluorescent Markers

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Description:	This literature review examines the available professional literature regarding the use of ATP bioluminescence and fluorescent markers to monitor the effectiveness of decontamination of the healthcare environment and reusable non-invasive patient care equipment.
Purpose:	To inform the existing and emerging technologies for decontamination of the health and care environment section on ATP bioluminescence and fluorescent markers.
Target Audience:	All staff involved in the prevention and control of infection in NHSScotland.
Update/review schedule:	Updated as new evidence emerges with changes made to recommendations as required. Review will be formally updated every 3 years with next review in 2026.
Cross reference:	National Infection Prevention and Control Manual (NIPCM)
Update level:	Practice – The implications for practice are updated based on a review of the extant scientific literature on ATP bioluminescence and fluorescent markers to monitor the effectiveness of decontamination of the healthcare environment and reusable non-invasive patient care equipment. Research – This review calls for further research into ATP bioluminescence and fluorescent marker methods and the cost effectiveness of these systems.

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Version history

This literature review will be updated in real time if any significant changes are found in the professional literature or from national guidance/policy.

Version	Date	Summary of changes
2.0	June 2023	This literature review replaces the Literature Review and Practice Recommendations: Monitoring the effectiveness of decontamination of the healthcare environment - ATP Bioluminescence and Fluorescent Markers, version 1.0 May 2017 and has been updated using the National Infection Prevention and Control Manual (NIPCM) two-person systematic methodology.
1.0	May 2017	Final for publication

Approvals

Version	Date Approved	Name
2.0	May 2023	Infection Control in the Built Environment and Decontamination Working Group (ICBED) Community Infection Prevention and Control Working Group (CIPC)
1.0	May 2017	ICBED Working Group

Contents

1. Objectives

The aim is to review the extant scientific literature regarding the use of ATP bioluminescence and fluorescent markers to monitor the effectiveness of decontamination of the healthcare environment and reusable non-invasive patient care equipment to form evidence-based recommendations for practice.

The specific objectives of the review are to determine:

- What is the actual or proposed mechanism of action of ATP bioluminescence and fluorescent marker monitoring systems?
- What is the procedure for using ATP bioluminescence and fluorescent marker monitoring systems?
- Are ATP bioluminescence and fluorescent marker monitoring systems currently used in health and care settings?
- What is the scientific evidence for effectiveness of ATP bioluminescence and fluorescent marker monitoring systems for monitoring decontamination of the healthcare environment?
- When should ATP bioluminescence and fluorescent marker monitoring systems be used in health and care settings?
- Are there any safety considerations associated with using ATP bioluminescence and fluorescent marker monitoring systems in the healthcare setting?
- Are there any practical or logistical considerations associated with using ATP bioluminescence and fluorescent markers in the healthcare setting?
- What costs are associated with using ATP bioluminescence and fluorescent markers in the healthcare setting?

2. Methodology

This targeted literature review was produced using a defined two-person systematic methodology as described in the National Infection Prevention and Control Manual: Development Process. Supplementary sections to the applied methodology for this specific literature review can be found in Appendix 2.

3. Discussion

3.1 Implications for practice

What is the actual or proposed mechanism of action of ATP bioluminescence and fluorescent marker monitoring systems?

ATP bioluminescence monitoring systems

The contaminated health and care environment is associated with the transmission of pathogens therefore, methods to assess the effective decontamination of surfaces can be considered an important part in the prevention and control of healthcare associated infections (HAIs).[1, 2] Among these methods include visual assessment, microbial methods, fluorescent markers and adenosine triphosphate (ATP) bioluminescence. ATP is an organic molecule present in all plant and animal matter including most food debris, bacteria, fungi and other microorganisms. It is necessary for transporting chemical energy within cells for metabolism. ATP can be used as an indicator of organic soil (for example shed skin cells, microorganisms) on surfaces. ATP reacts with firefly luciferase enzyme to produce light which is measured using a luminometer. [3, 4] This reaction is linear and one relative light unit (RLU) is equivalent to one molecule of ATP, for example the light emitted is directly proportional to the amount of ATP. The luminometer detects total ATP from both microbial and non-microbial sources in the sample including organic matter, bacteria, fungi, yeast and mould.[3-5]

Fluorescent marker monitoring systems

Fluorescent markers such as fluorescent gels are clear and colourless substances that fluoresce under blacklight/ultraviolet (UV) light. They were designed for the purpose of marking surfaces prior to cleaning and once applied they typically become invisible or transparent when dried and resist abrasion.[5] The fluorescent marker method does not measure the actual cleanliness of surfaces, it only indicates that the applied substance was physically removed through the assessment of the residual fluorescent substance after cleaning using blacklight or UV light.[5-7] By placing these products on surfaces in health and care settings before cleaning

takes place, the effectiveness of cleaning can be inferred by assessing whether or not the mark has been removed.[5, 7]

What is the procedure for using ATP bioluminescence and fluorescent marker monitoring systems?

A total of 23 low quality studies were identified relating to this topic appraised as SIGN50 level 3 and 4. The evidence included one guidance document from the United States of America (USA) Centres of Disease Control and Prevention (CDC) (2010), four manufacturer's (3M, Hygiena LLC, Kikkoman, Ecolab), operating manual and website instructions on how to use their branded ATP swab devices, luminometer and fluorescent markers and 17 primary studies.[4-26] Five studies provided evidence on the procedure for fluorescent markers while 22 studies were related to ATP bioluminescence method. The following ATP systems consisting of luminometers, and their consumable swabs were used by the included studies: 3M Clean-Trace ATP system (Clean-Trace NGi); Hygiena (Ultrasnap swabs and SystemSURE II/Plus), Kikkoman (Lumitester PD and LuciPac pen swabs), Charm novaLUM ATP system (Pocket Swab Plus), Lumicontrol II, and Accupoint Healthcare monitoring system. For fluorescent marker method, the following products were used in the included studies: Glitterbug, DAZO, Glo Germ Gel, DigoGlo and The Inspector.

Procedure for using ATP bioluminescence monitoring systems

ATP monitoring systems are designed to be a simple tool for measuring the level of ATP in a sample.[4, 24-26] The evidence is consistent regarding the general procedure for using the ATP bioluminescence method which involves sampling a standardised surface area with a specialised swab which is then analysed with a luminometer. [4-6, 8-26] The ATP systems consist of a flexible single-use pre-moistened swab contained within a sealed tube and a device for detecting and measuring bioluminescence (a luminometer). To perform the test the pre-moistened swab is removed from its sealed tube and allowed to equilibrate to room temperature (21 – 25 °C) before use.[24-26] A standardised area is thoroughly swabbed in one direction, then the other direction using a zig-zag or crisscross pattern while rotating the swab to maximise sample collection. The swab including the shaft should not be touched to avoid contamination of the swab test device.[24, 25] The swab is then replaced in its tube, at this point an activating reagent is released from within the tube by puncturing the compartment it is stored in

(this is typically done by clicking or snapping the top of the tube). The tube is shaken to mix the reagents, after the required time (typically 5 seconds) the ATP swab device is placed immediately into the luminometer which provides a result expressed in relative light units (RLU), typically in less than 30 seconds. There may be brand-specific instructions on how to activate the swab with the reagent therefore throughout the procedure manufacturer's instructions should be followed. After the test is complete, the swab device is removed from the luminometer and disposed of appropriately. The sampling area for swabbing with ATP testing devices is not standardised therefore there were inconsistencies in the identified evidence. Only one system provided specific instruction in its operating manual to swab a 10 x 10 cm (100 cm^2) or 4 x 4 inch area for a typical flat surface (for example tables and mattresses).[4, 24] The majority of studies (n = 12 papers) are consistent in swabbing a sampling area measuring 10 x 10 cm (100 cm^2).[6, 12-16, 18, 20-22, 24] Four studies sampled surfaces measuring 5 x 5 cm (25 cm^2) [8, 10, 11, 17] while three studies sampled surfaces measuring 10 cm^2.[9, 19, 23] For irregular surfaces (for example door handles), it is recommended to swab the area with consistent technique and pressure and to swab a large enough area to collect a representative sample.[11, 15, 24, 25] It is important to note that different ATP bioluminescence monitoring systems may have different design features and capabilities including software for analysing data. These features should be evaluated to determine whether it meets the needs of the intended application.

Procedure for using fluorescent marker monitoring systems

Fluorescent marker systems are designed to be simple to use and require minimal equipment. There is consistency among one CDC guidance document and 5 primary sampling studies that fluorescent clear marker products (e.g. fluorescent gels) are applied to surfaces, for example a high-touch surface, before cleaning.[5, 6, 17, 18, 27, 28] After cleaning, a blacklight (UV light) is shone on the area where the mark was placed to determine whether the mark was removed (successful cleaning) or not (cleaning unsuccessful).[5-7, 17, 18] Only 2 papers provided further information on the amount of fluorescent marker to be applied on surfaces with both suggesting marking surfaces with approximately 1 cm diameter of fluorescent marker.[6, 17]

Are ATP bioluminescence and fluorescent marker monitoring systems currently used in health and care settings?

A total of 25 studies were identified relating to this topic which included two United Kingdom (UK) guidance documents on best-practice cleaning specifications applicable to NHS Scotland (National Cleaning Services Specifications 2016) [29] and NHS England (National Standards of Healthcare Cleanliness 2021). [30] The remaining evidence were sampling studies graded as SIGN50 level 3 evidence (n=21 studies) [6, 8-14, 16-23, 27, 28, 31-33] and one SIGN50 level 4 report by Health Facilities Scotland (2016).[3] All scientific studies were conducted primarily in hospital settings such as medical wards, surgical wards, intensive care units (ICUs), operating theatres (OR), nursing homes and an ambulatory care setting. Most were carried out in the USA (7/22),[10, 11, 17, 18, 27, 28, 33] four were performed in the UK,[19-21, 23] three in Italy,[12, 13, 16] Brazil,[9, 22, 31] and Taiwan [6, 14, 32] and one in the Netherlands [8] and Sweden.[15]

The NHSScotland National Cleaning Services Specification sets out the cleaning specifications for NHS boards in Scotland however there was no mention of ATP bioluminescence or fluorescent marker monitoring systems.[29] Similarly, there was no mention of either method to monitor the effectiveness of decontamination in NHS England's National Standards of Healthcare Cleanliness (2021) although the guidance recommends that consideration should be given to audit technologies using objective methodologies to support the subjective measurement and efficacy of the cleaning process.[30]

Only two studies have mentioned using ATP bioluminescence method routinely to monitor hospital cleanliness: Health Facilities Scotland's (HFS) report on a visit to the North Tees NHS Trust (2011),[3, 15] and Knape et al (2015) reported that ATP bioluminescence method is used in Sweden to complement visual assessment for monitoring hospital cleanliness where it is now a widely accepted quality control standard within cleaning specifications required of hospital cleaning contractors.[15] Similarly, HFS (2011) reported that ATP bioluminescence method has been routinely used in the North Tees NHS trust to monitor hospital cleanliness but as part of a wider range of measures on decontamination of environment and equipment. ATP monitoring had been used effectively as 'a platform for promotion of hospital hygiene', to support training of domestic staff and as a process for performance management.[3] Both studies consistently state that ATP bioluminescence method was not used as a standalone measure for monitoring hospital cleanliness.

Although the efficacy of ATP bioluminescence and fluorescent marker monitoring methods have been investigated in numerous scientific studies, available literature suggests they are not routinely used as a standalone measure to monitor cleanliness in health and care settings.

What is the scientific evidence for effectiveness of ATP bioluminescence and fluorescent marker monitoring systems for monitoring decontamination of the healthcare environment?

A total of 28 studies were identified on this topic; all evidence were graded as SIGN50 level 3.[6, 8-14, 16-23, 27, 28, 31-40] No high quality studies such as randomised controlled trials (RCTs) were found on the topic of effectiveness of ATP bioluminescence and fluorescent marker systems for monitoring decontamination of the health and care environment. All 28 studies investigated efficacy of ATP method which included five studies related to the fluorescent marker method.[6, 17, 18, 27, 28]

Twenty-one studies were conducted in hospital settings including intensive care units (ICUs), medical and surgical wards, nursing home, operating rooms (OR) or operating theatres, outpatient clinics and emergency department (ED) [6, 8-14, 16-23, 27, 28, 31-33] while six were performed under laboratory and/or controlled experimental conditions.[34-40] In one study, assessments were performed on hospital surfaces and also under laboratory conditions.[39]

The cleaning methodology and products used in these studies varied widely and was typically poorly reported. Nine studies used disinfectants in their cleaning protocol including various concentrations of chlorine-based products (500-600 parts per million [ppm], 1000 ppm, 1080 ppm, 0.06% - 2% chlorine), quaternary ammonium-based or phenol-based products,[6, 10, 11, 14, 17, 18, 28, 32, 39] four studies used a combination of detergent and disinfectant products,[12, 13, 22, 31] five studies did not report this [8, 9, 23, 27, 33] and in two controlled laboratory-based studies, disinfection was carried out using vapourised hydrogen peroxide and steam/autoclave.[35, 36] Only two studies, both based in the UK, used detergents only for daily cleaning [19, 21] while another UK study used a detergent for routine cleaning of non-infected ward areas and 0.6% hypochlorite for wards housing patients with methicillin resistant *Staphylococcus aureus* (MRSA) but did not break down the results according to cleaning methodology.[20]

Eight studies monitored the surfaces after cleaning,[6, 16, 17, 21, 23, 34-36] one before,[33] 15 both pre and post cleaning [9-14, 18-20, 22, 27, 28, 31, 32, 39] and in one study, surfaces were sampled at random

points during the day independent of cleaning rounds.[8] A number of different commercially available systems were used across the studies (3M Clean-Trace [6, 8, 10-14, 17, 18, 20-22, 28, 31-38, 40] Hygiena system,[19, 23, 38, 40] Kikkoman [38, 40] Charm systems,[38, 40] Lumicontrol II[16] and one the ATP swab/device was not mentioned). [9] With regards to fluorescent markers, three of the studies assessed fluorescent marker removal using DAZO (Ecolab) [27, 28] and Glitterbug (Brevis) [6] only and two used a variety of markers [17, 18] (Glo Germ gel, Glo germ and DigiGlo, (Ecolab) Glitterbug (Brevis) or The Inspector (Creative solutions).

Different ATP benchmarks were used by the studies to discern between clean and dirty surfaces with < 250 RLU being the most commonly used cut-off value for surfaces classed as clean. The 250 RLU cut-off value was used by 11 studies, followed by <500 RLU (n=5 studies), <100 RLU (4 studies) and 2 studies used internal RLU values. To facilitate comparisons between studies RLU measurements have been converted to RLU/100cm^2 to account for any difference in size of sampling areas between studies.

Evidence for effectiveness of ATP bioluminescence monitoring systems

A total of 22 studies assessed the effectiveness of ATP bioluminescence assay compared with microbial methods for assessing effectiveness of environmental decontamination, typically of frequently touched surfaces. Eleven studies evaluated the correlation between ATP assay and microbial methods,[8, 12-14, 16, 22, 23, 31, 32, 37, 39] in six studies correlation was not investigated,[19-21, 27, 28, 33] two studies performed coefficient of covariance analysis,[18, 40] two studies carried out concordance analysis,[6, 11] and one study measured percentage reduction analysis of CFU and RLU results.[10]

Eleven primary studies investigated the correlation between RLU and aerobic colony counts (ACCs) or colony forming units (CFUs). In eight of these studies , a variety of frequently touched surfaces were sampled using ATP assay and ACC/CFU methods before and after daily or terminal cleaning with predominantly a combination of detergent and disinfectant products (quaternary ammonium compounds, chlorine-based products or 12.4 % glucoprotamin and alkyl dimethyl benzyl ammonium chloride products)[12-14, 16, 22, 31, 32, 39] while in one study, sampling of fomites was performed at random points during the day [8] and in two studies the cleaning or disinfection protocol was not reported.[23, 37]

Among the literature identified, there was consistent evidence from eight hospital-based studies showing no correlation or poor correlation between RLU and ACCs/CFUs (Pearson's correlation or Spearman's correlation coefficient r = 0.0018 to 0.29) suggesting that when there is a decrease in ACC after cleaning, it was not possible to assume a decrease in ATP results and vice versa.[8, 12-14, 16, 23, 31, 39] In other words, the level of light emitted by the ATP bioluminescence method is not directly related to the number of viable microorganisms in the sample. Based on this evidence, ATP assay does not provide an accurate or direct measure of viable microbial contamination on environmental surfaces sampled.

Of the 11 studies, two evaluated RLU and microbial analysis (ACC or CFU) monitoring methods of surface disinfection in operating rooms (OR) before and after cleaning. [11, 12] The two studies used different ATP threshold values of 100 RLU[12] and 250 RLU[11] for clean/pass despite using the same ATP system. In one study of ORs from two Italian hospitals, the microbial contamination of frequently touched surfaces (medical anaesthesia trolley, nurse's computer touch screen, operating table, vitals monitor, anaesthetist's computer touch screen, surgical lighting, and instrument table) was assessed with ATP method and compared with microbial analysis (CFU); an ATP value of 100 RLU/100cm^2 & < 15 CFU/plate was used as benchmark.[12] Surfaces were sampled using both methods before and after turnaround cleaning using a detergent and disinfectant (active chlorine 1080 ppm) solution. Turnaround cleaning in ORs refers to the process of cleaning and preparing an OR for the next surgical procedure after the previous procedure has been completed and typically involves cleaning and disinfection of surfaces. Of 140 sampled surfaces, 120 (85.7%) had concordant results, of these 119 were within limits of both ATP and CFU methods and one exceeded both limits. No statistically significant correlation between ATP (RLU) and microbiological data (Pearson's test r = 0.169; P = 0.046) was found suggesting ATP results cannot be interpreted as an indicator of microbial contamination. Despite this, the two methods were consistent in identifying the most contaminated surface (surgical lighting). This study demonstrates that ATP bioluminescence method may not be an appropriate replacement for culture method when it comes to evaluating microbial contamination in the health and care environment.[12] Furthermore, a study conducted in the USA evaluated the effectiveness of the ATP method and the replicate organism detection and counting (RODAC) assay in assessing the cleanliness of irregularly shaped surfaces (overhead lights, door handles, and anaesthesia keyboards) in comparison to regularly shaped/flat surfaces (mattresses and side tables) in 24 operating rooms.[11] Sampling was performed before and after turnaround cleaning between procedures using either quaternary

ammonia with microfiber cloths and/or disposable bleach disinfectant wipes. Concordant results between RLU and CFU were observed for only 65% (75/120) of surfaces sampled. Irregularly shaped surfaces were more likely to fail by ATP assay both before and after cleaning whereas they were more likely to pass by RODAC assay than ATP assay after cleaning.[11] Study findings suggest that irregular shape and increased surface area may make these surfaces more prone to contamination and harder to clean however, the reasoning for this is inconclusive.

Overall findings from these two OR studies show no significant correlation or low concordance of results between ATP bioluminescence assay and microbial methods suggesting ATP levels are not related to the results obtained from microbial methods even though both tests are used to measure microbial contamination. Although ATP method can identify 'dirty' or contaminated surfaces, it measures total bioburden and cannot distinguish between pathogenic and non-pathogenic microorganisms or even live-versus-dead cells therefore there is insufficient evidence to determine its use to monitor the effectiveness of decontamination in operating rooms. [11, 12]

In contrast, only three studies were identified that showed significant correlation between RLU and culture-based methods although the correlation was moderate ranging from r = 0.47 to 0.879.[22, 32, 37] In one of these studies, five frequently touched surfaces (dressing trolley, stretcher, reception desk, outpatient support table and outpatient operating table) were sampled before and after routine daily cleaning using a combined detergent and disinfectant product containing 12.4% glucoprotamin and 15% alkyl-didimethyl- benzyl-ammonium chloride.[22] Among the 120 surfaces evaluated before cleaning and disinfection, 49.1% and 45% were considered dirty according to ACC and ATP bioluminescence respectively versus 12.5% and 16.6% after cleaning and disinfection. There was a significant correlation (Spearman's correlation coefficient) for 2 surfaces only: reception desk (r = 0.598, P=0.002) and stretcher (r = 0.422; P=0.04) while no correlation was found for the other 3 surfaces (r = 0.051 to 0.149; all P>0.05). [22] Evidence from this study is limited by the small number of surfaces sampled and in addition, the ATP device, cleaning and disinfection protocol and disinfectant product used at this hospital may differ from those in other studies preventing generalisation of results. In another hospital-based study, the effectiveness of daily cleaning using 0.06% sodium hypochlorite (NaOCl) and 0.05% sodium dichloroisocyanurate (NaDCC) was assessed by sampling 11 frequently touched surfaces (bedside rails, bedside tables, chairs, doorknobs, drawer handles, emergency buttons, light switches, hand sanitizer pump, toilet flush handles, toilet safety rails and wardrobe handles) using both ATP and ACC methods. Adjusting for

sampling area, a significant but moderate correlation was observed between ATP and culture-based method (r = 0.47; P<0.001). The authors estimated using a ROC curve analysis that the best ATP cut-off point was 7.34 RLU/cm^2 or 734 RLU (sensitivity 74% and specificity 67%).[32] Lastly, a laboratory-based study evaluated ATP method efficacy in determining microbial contamination on 17 sterilised coupons from surfaces commonly found in hospital environments, and whether the ATP measurements of *Acinetobacter baumannii*, *Candida albicans*, *Enterococcus faecalis, Escherichia coli*, *Mycobacterium smegmatis*, and MRSA correlated to culture-based method (CFU).[37] There was a significantly strong correlation between log-adjusted CFU and RLU measurements across each of the 17 test surfaces for each microorganisms tested (all P<0.05) with the exception of *S. aureus* tested on paper (Pearson correlation = 0.570, P=0.109). Combining all organisms and concentrations (~10^4, 10^6, 10^8 CFU/surface) demonstrated a significant correlation of 0.879 (P<0.001). Evidence from this study should be treated with caution due to the following important limitations: the study was conducted under controlled laboratory conditions using pure cultures and there was no adjustment for ATP biological contaminants that may be found in the healthcare environment.

Additionally, no significant correlation was observed in two laboratory-based studies that compared ATP and CFU results after decontamination of surfaces with vaporised hydrogen peroxide (VHP) and steam/autoclave.[35, 36] Stainless-steel coupons were inoculated with known amounts of organisms including *Acinetobacter baumannii*, *Bacillus anthracis* Sterne endospores, *Bacillus anthracis* Sterne vegetative cell, *Candida albicans* wild type, *Clostridioides difficile* wild type, *Escherichia coli*, *Enterococcus faecalis*, MRSA, *Klebsiella pneumoniae*, *Geobacillus stearothermophilus* and *Mycobacterium smegmatis* then disinfected over set time periods with either steam or VHP. This resulted in an observed reduction of CFU but no corresponding reduction in RLUs. Furthermore, a live versus dead experimental design yielded an expected 100% microbial kill result for quantitative microbiology after passage through an autoclave; all post-autoclave CFU counts were zero for all organisms. However, ATP bioluminescence method detected changes in ATP levels between pre-exposure and post-exposure to steam sterilisation/autoclave with variable remaining percentages for microorganisms ranging from 0 to 7%. The major exception was *S. aureus* which measured 70% of pre-autoclave value and *B. anthracis* vegetative cells which showed an 87% increase indicating more ATP was detected after autoclave versus before pre-autoclave.[35, 36] Findings from these two in vitro studies demonstrate that ATP bioluminescence assay was incapable of distinguishing between viable organisms, non-viable organisms and organic debris. This

suggests that for the organisms tested, ATP testing was not an ideal replacement for plate counts to determine microbial inactivation after decontamination with VHP or steam. ATP readings may provide false positives and therefore prompt unnecessary need to re-clean surfaces.

Sensitivity and specificity of ATP bioluminescence method

Sensitivity refers to the accuracy of the test to detect low levels of ATP in a sample while specificity refers to the ability to accurately identify ATP from other substances that may be present in the sample. The sensitivity and specificity of ATP bioluminescence method relative to the gold standard ACC was measured in eight hospital-based studies .[6, 13, 14, 17, 19, 27, 28, 31] The sensitivity of ATP bioluminescence ranged from 20% to 78% and specificity at 38% to 100%. A study assessed ATP and ACC/cm^2 before and after detergent cleaning in one surgical and one medical ward in a Scottish hospital.[19] A total of 90 surfaces were sampled (bedside locker, bedframe left side, overbed table, floor under bed, bedframe right side, bedside curtain, patient notes, computer keyboard, nurses' desk and toilet door pushplate) with Hygiena system. The study provided 270 paired ATP and microbial readings. There was weak evidence for ATP as an indicator for microbial growth and results indicated that a cut-off value of 100 RLU was optimum (sensitivity 57%, specificity 57%). It should be noted that this ATP benchmark infers the use of the Hygiena system. A further six hospital-based studies calculated sensitivity and specificity for ATP bioluminescence using 3M Clean-Trace system with two studies performed in the USA, two in Taiwan, one in Brazil and one in Italy.[6, 13, 14, 17, 28, 31] There was some variability in the cleaning methodology, types of frequently touched surfaces and sampling strategy used in these six studies. The sensitivity of ATP bioluminescence was calculated as 20% to 70.3% while specificity was reported as 42.9% to 100%. Finally, a community-based hospital study from the USA using Accupoint Healthcare monitoring ATP system sampled a total of 250 frequently touched surfaces (call button, telephone, bedrail, table and toilet rail) before and after terminal cleaning (cleaning products not reported) using ACC cut-off ≤2.5CFU/cm2 and ATP cut-off of < 250 RLU.[27] Before cleaning, 53% of surfaces were classed as clean by ATP assay and 59% by ACC. After cleaning, 76% of surfaces were classed as clean by ATP assay and 87% by ACC. Compared to ACC, ATP found significantly fewer surfaces considered clean after terminal cleaning ($p<0.001$). The sensitivity of ATP to detect clean surfaces was 78% and the specificity was 38%.

In summary, there was wide variability in the reported sensitivity and specificity of ATP bioluminescence. It is challenging to compare the variable sensitivity and specificity reported by multiple studies due to variations in factors such as study design, sampling characteristics, cleaning methodology and ATP device used. Ideally, ATP bioluminescence should be sensitive enough to correctly identify even low levels of ATP on a sample as well as highly specific to identify the proportion of samples that do not contain ATP.

Evidence for effectiveness of fluorescent marker monitoring systems

This literature review identified five studies all of which were appraised as level 3 using the SIGN50 criteria.[6, 16, 17, 27, 28] All were hospital-based and they evaluated the effectiveness of using fluorescent marker removal to detect clean surfaces after terminal cleaning using the microbiological gold standard ACC as a comparison. Three of the studies used quaternary ammonium compounds for cleaning,[17, 18, 28] while one study used sodium hypochlorite.[6]
A fluorescent marker was applied to high touch surfaces before terminal cleaning and examined by UV light after cleaning. The criteria for determining clean surfaces varied among the studies. Three studies considered surfaces clean if they yielded ACC of <2.5 CFU/cm^2,[18, 27, 28] while in the remaining two studies, the cut-off for clean surfaces was set at <5 CFU/cm^2.[6, 17] Surfaces were considered clean if the fluorescent marker was completely or partially removed while in one study a plastic-circle visualisation tool was used to assess the amount of residual fluorescent gel. All five studies found that the proportion of surfaces considered clean using fluorescent markers was lower than using ACC and the range of sensitivity (40.4% to 68%) and specificity (50% to 80.3%) of fluorescent marker removal was generally poor.[6, 17, 18, 27, 28]
Findings indicate that the correlation between fluorescent marker removal and ACC was poor, with a tendency for the marker to falsely report a surface as dirty. The overall evidence suggest that fluorescent marker removal tools are not as effective as ACC in detecting clean surfaces after terminal cleaning.

In conclusion, evidence from the available literature reports varying sensitivity and specificity values for both ATP bioluminescence and fluorescent marker monitoring systems due to many factors including differences in the sample preparation, variations in the ATP and fluorescent devices used and variations in the conditions used during the assay. Additionally, the inherent variability of the ATP assay and fluorescent marker method can also contribute to the variations in results. Generally, both methods have poor sensitivity and poor specificity and are more likely to generate false positives (assessing a surface as ‘dirty’ that is microbiologically clean) than

false negative results. The sensitivity and specificity of ATP monitoring systems may be improved by following established protocols and internal validation of acceptable ATP limits against a microbiological comparator (CFU/cm^2).

There is insufficient evidence to support the use of either ATP bioluminescence or fluorescent marker monitoring systems to infer the microbiological cleanliness of a surface. However, both methods may be useful for monitoring purposes provided appropriate and local benchmarking is implemented.

Impact of detergents/disinfectants on ATP readings

One laboratory-based study investigated the effects of disinfectants on ATP readings. Quenching of ATP refers to the process by which the light emitted by the luminometer is reduced or 'quenched' by the presence of disinfectants while enhancement effect refers to an increase in the observed bioluminescence when certain disinfectants are present during the test. Omidbaksh et al (2014) evaluated the quenching and enhancement effect of 14 disinfectants on 4 commercial ATP bioluminescence monitoring systems: 3M, Hygiena, Kikkoman and Charm.[38] The test disinfectants included quaternary ammonium chlorides, phenol, sodium hypochlorite, isopropanol, citric acid and hydrogen peroxide. Ten µL of appropriate dilution of ATP standard solution was placed onto the ATP swab followed by 10 µL of test disinfectant. Disinfectants affected ATP readings across all 4 tested units with 3M-meter being the most susceptible to disinfectant chemistries. The majority of the tests demonstrated quenching effect, with phenol-based formulations showing highest quenching among all tested disinfectants. It was not possible to determine whether the interference was attributable to phenol, other active ingredients or other inert ingredients in the products. This laboratory-based study suggests that disinfectants may affect ATP readings due to high levels of residual chemicals potentially resulting in false negatives or false positive results. It should be noted that in this study, disinfectants were applied directly to the swab while in real clinical settings, disinfectants will be applied to the surface first and will most likely be dry before swabbing. Overall findings suggest that ATP bioluminescence is not a reliable disinfection validation tool however further high-quality research set in clinical settings is required to further confirm results.

When should ATP bioluminescence and fluorescent marker monitoring systems be used in health and care settings?

Three documents were identified on this topic consisting of two SIGN50 level 4 guidance from the CDC and NHS England and one SIGN50 level 3 evidence report from Health Facilities Scotland.[3, 5, 30] There was no mention of ATP bioluminescence and fluorescent marker monitoring systems in the NHSScotland National Cleaning Services Specification, a best-practice guidance for health and care staff in NHS Scotland.[29] There is consistency demonstrated by the limited evidence that organisations should consider objective tools to identify areas that are poorly decontaminated and these tools, which include ATP and fluorescent marker methods, can support the subjective measurement of the cleaning process. As seen in the previous section that assessed the effectiveness of both methods, there is insufficient high-quality evidence to support the use of ATP bioluminescence and fluorescent marker monitoring systems as a standalone measure to infer microbiological cleanliness of a surface. However, organisations could consider objective evidence-based tools such as ATP bioluminescence and fluorescent marking methods to complement the subjective measurement (for example, visual inspection) of the cleaning process.

Are there any safety considerations associated with using ATP bioluminescence and fluorescent marker monitoring systems in the healthcare setting?

No scientific study was identified on safety considerations associated with the use of either ATP assay method or fluorescent marker method. Hand searching yielded 2 manufacturer operating instructions, graded SIGN50 level 4; neither indicated any health risk when used in accordance with operating instructions. [4, 24]

Are there any practical or logistical considerations associated with using ATP bioluminescence and fluorescent markers in the healthcare setting?

This review found two SIGN50 level 4 graded evidence relating to this topic consisting of manufacturer operating manuals on ATP surface test devices.[24, 26] It is recommended by the manufacturer that ATP swab devices should be stored out of direct sunlight and within the recommended temperatures of 2 °C – 8 °C (36 °F – 46 °F). This suggests that long term storage of ATP swabs/kits may require the use of refrigerators to maintain the recommended temperatures. Additionally, some ATP devices have a specific shelf-life.[24] In the procedure described for North Tees Hospital Trust ATP monitoring is performed on cleaned rooms and no-one enters rooms between cleaning and ATP assessment, this could potentially be difficult in busy clinical areas.[3]

What costs are associated with using ATP bioluminescence and fluorescent markers in the healthcare setting?

Six pieces of SIGN50 level 4 evidence was found on costs associated with ATP bioluminescence methods however no formal cost-benefit analysis was available.[3, 20, 41-44] No evidence was found on fluorescent marker methods relating to this topic. ATP bioluminescence monitoring systems require the use of a luminometer device and consumables (swabs). Consideration should be given to the initial costs of purchasing a luminometer. Additionally, subsequent testing will require the use of additional ATP swabs therefore these costs should also be considered.[4, 24] A 2009 observational study by Sherlock et al reported the cost per test of an ATP swab as €2.80 while the time to result was 20 seconds. In comparison, ACC swab and plating was €0.60 with time to result being 48 hours.[20] It is important to note that these are consumable costs and do not include processing costs. The 2011 North Tees Hospital Trust report stated that each swab cost £1 and that 5 swabs were used per room. This did not include the initial cost of the luminometer(s).[3] As these costings were conducted over 10 years ago, valid comparisons cannot be made, keeping in mind the effect of inflation and cost rises. As of January 2023, 4 commercial company websites reported that a box containing 100 ATP swabs costs approximately £180.36 to £288.54 while ATP luminometer devices were priced between £1013.70 to £2,370.00 depending on the specification.[41-44] Performing ATP measurements

requires the additional consumable costs of swabs, along with processing, which can be a limiting factor for using ATP measurement in Scottish health and care settings.

3.2 Implications for research

This review identified persistent research gaps in this topic area. Firstly, as demonstrated in this review there is a lack of consistency in established acceptable limits for ATP readings that indicate that a surface is clean. Most ATP bioluminescence kits rely on the use of swabs to sample surfaces however there is no standardised protocol for swabbing particularly irregularly shaped surfaces. Further studies are required to determine the most effective but simple to follow sampling method(s). The majority of included studies achieved a low-quality rating when assessed with SIGN50 methodology and there is very limited published evidence on the effectiveness of fluorescent marker monitoring systems. Further studies are required with consistent and clearly presented methodology. Although some studies demonstrated that ATP results correlate with aerobic colony counts on tested surfaces, many more studies have not. Additional studies are required to determine if low ATP readings correlate with reduced surface contamination and therefore lower risk of HAIs. There is an issue with clear reporting of cleaning methodology used in the included studies which should have been addressed during peer review before publication. It will also be necessary to elucidate in studies set in health and care settings whether some products (detergent/disinfectants) may interfere with the ATP assay itself and in what direction this may influence results. Finally, there was limited assessment of the in-use costs associated with the use of routine monitoring with ATP and/or fluorescent marker method.

4. Recommendations

This review makes the following recommendations based on an assessment of the extant scientific literature on ATP bioluminescence and fluorescent markers to monitor the effectiveness of decontamination of the health and care environment and reusable non-invasive patient care equipment.

What is the procedure for using ATP bioluminescence and fluorescent marker?

For ATP Bioluminescence method:

For typical flat surfaces (for example tables and mattresses), a standard 10 x 10 cm (100 cm^2) or 4 x 4-inch area should be thoroughly swabbed in a zigzag or crisscross pattern while applying consistent pressure.

(Category C recommendation)

For irregular surfaces (for example door handles and bed rails), the area should be swabbed with consistent technique and pressure, covering a large enough area to collect a representative sample.

(Category C recommendation)

For fluorescent marker method:

Fluorescent markers should be applied to surfaces before cleaning and assessed with a blacklight or UV light after cleaning to determine whether the mark was removed (successful cleaning) or not (cleaning unsuccessful).

(Category C recommendation)

There may be brand specific instructions for both ATP bioluminescence and fluorescent marker methods therefore manufacturer's instructions should be followed throughout the procedure.

(Category C recommendation)

Are ATP bioluminescence and fluorescent marker monitoring systems currently used in health and care settings?

Available literature suggests that ATP bioluminescence and fluorescent marker monitoring systems are not routinely used as a standalone measure to monitor cleanliness in health and care settings.

(No recommendation)

What is the scientific evidence for effectiveness of ATP bioluminescence and fluorescent marker monitoring systems for monitoring decontamination of the healthcare environment?

ATP bioluminescence or fluorescent marker monitoring systems may be used to assess bioburden as an adjunct to training however there is insufficient evidence to support the use to infer microbiological load or cleanliness of a surface.

(Category C recommendation)

When should ATP bioluminescence and fluorescent marker monitoring systems be used in health and care settings?

ATP bioluminescence and fluorescent marker monitoring systems can be used to monitor cleaning compliance in the health and care environment.

(Grade C recommendation)

Where ATP bioluminescence or fluorescent marker monitoring systems are used, appropriate local benchmarking, methodology and practice should be implemented prior to use.

(Category C recommendation)

Staff using ATP bioluminescence and fluorescent marker monitoring systems must be fully trained in the product's use/limitations.

(Category C recommendation)

Are there any safety considerations associated with using ATP bioluminescence and fluorescent marker monitoring systems in the healthcare setting?

No safety risks have been identified with the use of ATP bioluminescence monitoring systems including ATP swabs and luminometer devices or fluorescent marker monitoring systems when used in accordance with manufacturer's instructions.

Manufacturer's instructions should be followed when using ATP bioluminescence and fluorescent marker monitoring systems.

(Category C recommendation)

Are there any practical or logistical considerations associated with using ATP bioluminescence and fluorescent markers in the healthcare setting?

ATP bioluminescence assay consumables e.g., ATP swabs should be stored out of direct sunlight and at low temperatures (for example 2 °C to 8 °C) according to manufacturer's instructions.

(Category C recommendation)

Some ATP swab devices may have a specific shelf-life therefore the expiration date on the label should be referred to.

(Category C recommendation)

What costs are associated with using ATP bioluminescence and fluorescent markers in the healthcare setting?

ATP bioluminescence monitoring systems require the use of a luminometer device and consumables (ATP swabs). Consideration should be given to the initial costs of purchasing a luminometer and ongoing consumable costs (ATP swabs).

(Category C recommendation)

There was insufficient evidence to inform about the costs associated with using fluorescent markers in the health and care settings.

(No recommendation)

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Appendix 1: Grades of Recommendation

Grade	Descriptor	Levels of evidence
Mandatory	'Recommendations' that are directives from government policy, regulations or legislation	N/A
Category A	Based on high to moderate quality evidence	SIGN level 1++, 1+, 2++, 2+, AGREE strongly recommend
Category B	Based on low to moderate quality of evidence which suggest net clinical benefits over harm	SIGN level 2+, 3, 4, AGREE recommend
Category C	Expert opinion, these may be formed by the NIPC groups when there is no robust professional or scientific literature available to inform guidance.	SIGN level 4, or opinion of NIPC group
No recommendation	Insufficient evidence to recommend one way or another	N/A

Appendix 2: PRISMA Flow Diagram