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Assessing the effectiveness of hospital cleaning using fluorescence: a proof-of-concept study and comparison with ATP testing

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SUMMARY

Visual inspections are used to assess hospital cleanliness, as visible contamination may present health risks and influence perceptions of care quality. Problematically, many contaminants are invisible to the naked eye, limiting the reliability of visual checks. Many invisible substances, however, fluoresce (i.e. emit visible light after absorbing electromagnetic radiation). Portable torches can detect fluorescent substances *in situ*, offering a potential method to enhance cleaning practices.

This study has evaluated fluorescence as a tool for identifying general invisible contamination after hospital cleaning. Visibly clean surfaces in seven single-occupancy patient rooms and two six-bed wards across two National Health Service hospitals were examined using a portable high-intensity blue and ultraviolet light torch. Adenosine triphosphate (ATP) levels in fluorescent and non-fluorescent areas were taken as a recognized cleaning monitoring tool, and analysed statistically using Wilcoxon signed-rank tests.

Fluorescent contamination that was invisible to the naked eye was found on every surface. ATP relative light unit (RLU) levels were significantly higher in fluorescent substances compared with non-fluorescent substances ($P \leq 0.05$) with large effect sizes on patient chairs, bed frames, overbed tables, bedside units and pillows, but not toilets, sinks or commodes. The mean RLU measurement was 161 in fluorescent areas compared with 33 RLU in control areas.

Fluorescence detected alternative contamination which could present toxic risk to humans, such as cleaning fluid and/or drug-contaminated residue which frequently contain fluorescent constituents. This was an important finding as relying solely on ATP detection may overlook significant contamination risks. Further work to evaluate the method as a cleaning aid is encouraged.

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Introduction

Thorough and effective cleaning plays a critical role in protecting patients, staff and visitors by removing pathogens, chemical residues, and hazardous debris.[a] A visibly clean environment is not only essential for physical safety, but also significantly influences perceptions of care quality.

Adherence to cleaning standards set by national health authorities and accreditation bodies is a mandatory requirement for hospitals. Regular cleaning audits – guided by frameworks such as the National Standards of Healthcare Cleanliness [1,2] and international equivalents [3–5] – are designed to ensure compliance. Despite their importance, these audits often rely heavily on visual inspections, which are inherently subjective and cannot detect contamination that is invisible to the naked eye. Cleanliness is assumed based on the application of approved cleaning protocols and the absence of physical matter, yet microbial and chemical contaminants may reside even when surfaces appear clean. Research has shown that pathogens found in invisible contamination, such as body fluids, non-intact skin and mucous membranes [6], can survive for weeks or months on inadequately cleaned surfaces [7,8].

Numerous studies have highlighted the need for more reliable and objective methods to assess hospital cleanliness [9–11]. While supplementary tools such as adenosine triphosphate (ATP) testing and microbial analysis may supplement visual inspections, they are not without limitations. ATP levels do not always correlate with infection risk or microbial presence [12,13], and microbial investigation requires specialist equipment and expertise that takes time to deliver results. It is impractical to sample a hospital environment exhaustively due to cost and time, so critical, high-touch areas are typically targeted. Consequently, contamination can easily be missed and therefore the test result may not reflect the wider environment accurately.

Enabling cleaning staff to see invisible contamination during cleaning would logically provide a cleaner environment. An underexplored technique involves the use of naturally occurring fluorescence [i.e. light emitted by a wide variety of substances such as body fluids and drug residues when exposed to some types of electromagnetic radiation, including ultraviolet (UV) and high-intensity blue light]. This method is widely used in forensic science work [14–16], using small, portable torches that are simple to use and can easily be taken to crime scenes where they are used to detect invisible substances. Although UV light has been used in hospitals for cleaning audits, this has typically involved the application of fluorescent gel markers in advance to assess thoroughness [17–19]. However, these markers are artificially designed to fluoresce, and require only low-intensity light to activate. In contrast, identifying naturally fluorescent contamination requires higher-intensity lighting. This approach, used in accordance with stringent health and safety assessments, may assist in both the direct removal of contaminants and the identification of high-risk sites for further microbiological or ATP testing.

The aim of this study was to explore the potential of fluorescence to support cleaning work using portable torches that are used in forensic work, working in partnership with the infection prevention and control team and facilities management leaders who oversee cleaning operations. Part of the evaluation involved a comparison with ATP testing, as a widely adopted method for monitoring cleaning performance, although the detection of alternative contamination was equally relevant to the study.

[a] In this paper, the term 'contamination' is used to define all hazardous infectious agents, hazardous chemicals, or physical items that may compromise patient safety, such as clinical waste.

Methods

An experimental field study was carried out in seven single-occupancy patient rooms and two six-bed wards across two National Health Service (NHS) hospitals. All rooms were cleaned by in-house domestic staff using Actichlor Plus and microfibre cloths, in accordance with the National Standards for Cleaning (NHS Wales) [1], following patient discharge.

NHS staff conducted visual inspections of 39 items, each of which was recorded as 'visibly clean'. Surfaces were then examined using either a Class 2 blue light torch (450-nm peak wavelength, filtered at 578 nm; Foster and Freeman Ltd, Evesham, UK) or a Class 3 blue light torch (455-nm peak wavelength, filtered at 578 nm[b]; CopperTree Forensics Ltd, Leatherhead, UK), and a UV light torch (365-nm peak wavelength with UV filter; Foster and Freeman Ltd).

Fluorescent contamination was photographed *in situ* with a digital single lens reflex camera and a 578-nm camera filter. The outcome was binary (fluorescent or not) based on whether or not the researcher detected fluorescent contamination.

On 14 high-touch patient items included in the health board's standard operating procedure (SOP) for ATP testing, 197 fluorescent substances were swabbed using SureSnap swabs (Hygiena, Camarillo, CA, USA) and assessed with a EnSure Touch luminometer (Hygiena), providing relative light unit (RLU) data. Non-fluorescent (control) areas (10 cm^2) immediately next to the fluorescent substance were then swabbed (using a plastic scale). According to the SOP, any reading $\leq$50 RLU was classified as 'clean'. Due to financial and logistical constraints, not all fluorescent samples could be tested for ATP; the volume of fluorescent substances exceeded available resources. The number of each item varied according to the size of the item and the number of fluorescent substances found (larger items such as bed frames generally had more than a handrail). Seventy-three, non-fluorescent control samples were taken as baseline measurements to infer whether the quantity of ATP was attributable to the fluorescent sample. On smaller items, such as a patient call bell, one control sample was taken because the swab covered a significant quantity of the item. Multiple control samples were taken on larger items to represent a wider surface area. For example, a bed frame.

RLU data from fluorescent (experimental) and non-fluorescent (control) sites were compared statistically using Wilcoxon signed-rank tests using SPSS Version 28 (IBM Corp.,

[b] This was to infer how a Class 2 torch compared with a Class 3 torch. The power output of a Class 3 device is greater than a Class 2 device, and therefore Class 3 devices may be more sensitive. A Class 3 device is more hazardous than a Class 2 device, and their use in the UK is strictly governed by The Control of Artificial Optical Radiation at Work Regulations (2010).

Armonk, NY, USA). An alpha level of 0.05 was applied and effect sizes were calculated accordingly:

$$r = z \Big/ (\sqrt{N})$$

where z = test statistic and N = sample size. A value of 0.1 indicated a small effect, 0.3 indicated a medium effect, and 0.5 indicated a large effect.

Results

Filtered blue and UV light rapidly detected imperceptible fluorescent substances that had not been removed during cleaning on every surface that was examined. The appearance and number of substances varied (Figure 1). None of these substances could be seen with the naked eye (all surfaces had passed a visual inspection).

With the naked eye, there was no discernible difference in the appearance of a stain that did and did not contain ATP (Figure 1). In 126 of 197 cases, the ATP content of fluorescent substances was higher than that of non-fluorescent samples, and the difference was significant [$P \leq 0.05$, very large effect size (0.2: Cohen's D)].

When the data were considered by item, significant differences ($P \leq 0.05$) in ATP with large effect sizes were found on patient chairs (effect size 0.7), bed frames (0.4), overbed tables (0.5), bedside units (0.5) and pillows (0.6). No significant differences ($P > 0.05$) were found on toilets, sinks and commodes, although fluorescent substances were found with high RLU values. For example, toilets yielded RLU values between 600 and 1200.

Of 197 fluorescent substances, 85 yielded ≥50 RLU (43%) and 21 ≥50 RLU (28%) of non-fluorescent areas were classed as 'unclean' in terms of ATP alone, as only ATP was tested for. All (100%) surfaces were classed as visible clean, but when using fluorescence as a measure, 100% of surfaces were considered to be 'unclean'. As part of routine environmental monitoring at the health board, six ATP test sites are selected at random from the SOP [1], suggesting that the method was better at locating ATP-containing substances than chance alone.

Most fluorescent contamination fluoresced under blue or UV light, but there were instances where blue light fluoresced matter that UV light did not, and vice versa. Figure 2 provides an example of fluorescent contamination on a bed frame under UV light (left) and filtered blue light (right). Area 'a' contains (likely) dust particles that did not fluoresce under blue light. Both lights detected 'b', and blue light detected further contamination in 'c'.

As described in the Methods section, two blue torches were used. There was no discernible difference in the quantity of fluorescent substances detected using a Class 2 torch compared with a Class 3 torch.

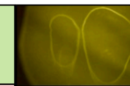
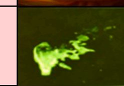
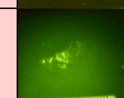
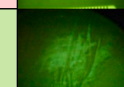
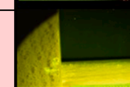
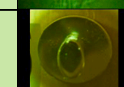
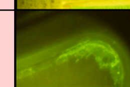
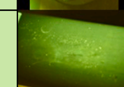
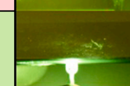
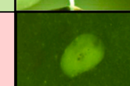
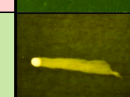
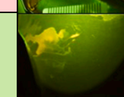
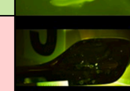
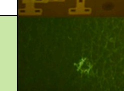
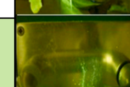
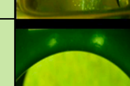
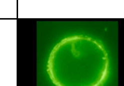
Mattress–brown		Toilet bin	
Bed head/foot		Bin (orange clinical)	
Enamelled bedframe		Toilet cistern	
Bed control		Mattress–blue	
Chair arm rest		Toilet door lock	
Overbed table		Handrail – toilet wall	
Curtain rail		Toilet seat (raised)	
Bedside cabinet (top–white)		Flush handle	
Bedside cabinet door		Sink splashback	
Patient call bell		Power socket (plastic)	
Sink		Power socket (stainless-steel)	
Tap		Chair (blue fabric)	
Soap and paper towel dispenser		Green blanket and white bedsheet	A, F.
Light switch		Toilet door (including panel overlay)	A.
Door handle		Handrail – next to toilet	F.
Commode[a]		Commode[b]	
Painted wall		Television and remote	A.
Pillow		Cable trunking	F

Figure 1. Fluorescence response data by item/surface. Items without images were not suitable for fluorescence examination due to inherent fluorescence (F) or surface absorbance (A). All surfaces shown with Class 3 blue light and 578-nm filter. Call bell shown under ultraviolet light. Items highlighted red = fluorescent contamination >50 relative light units (RLU) (unclean according to this threshold). Items highlighted green = fluorescent contamination ≤50 RLU (clean according to this threshold). [a]Physical surface contamination. [b]Likely fluorescent adhesive as part of manufacture.

Discussion

Fluorescence can quickly reveal physical residues that are not visible to the naked eye. The use of blue light and UV light collectively increased the amount of matter that was found because of the molecular composition of the substances, and

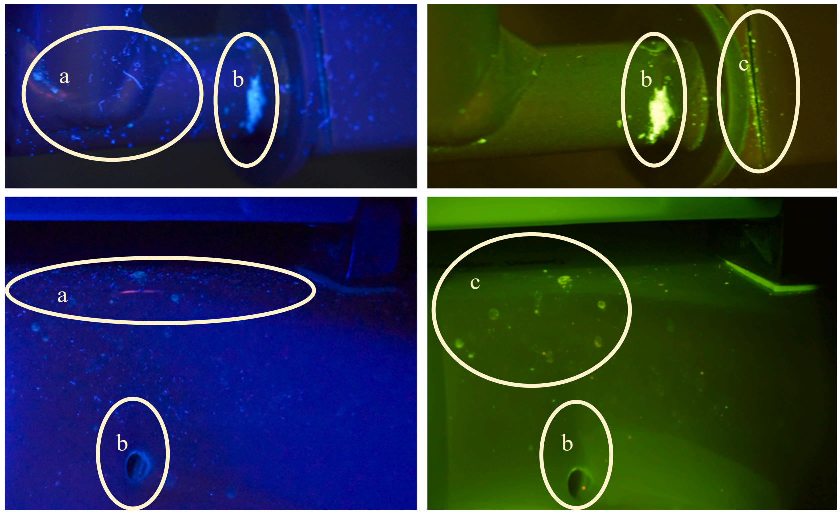


Figure 2. Differences in the fluorescent response of invisible contamination on surfaces that are suitable for fluorescence examination [left = ultraviolet (UV) light, right = Class 2 blue light and 578-nm filter]. Area a is visible under UV light alone. Area b is visible under both lights. Area c is visible under blue light alone.

how they absorb and emit light [20]. At present, the method cannot identify what the substance is, although in a hospital environment, the presence of a single fluorescent substance is unlikely and would require further, specialized analysis, such as Fourier transform infra-red spectroscopy (FTIR) or high-performance liquid chromatography, likely followed by mass spectroscopy, to identify and quantify its source. In a hospital environment, where cleaning is ongoing, similar to microbial testing, it is not practical nor financially viable to monitor an environment continually or exhaustively. This work has deliberately focused on a portable, simple-to-use method for screening larger areas which gives an instant result and requires no specialist interpretation.

Cleaning removes visible contamination based on a logical assumption that it contributes to a cleaner and safer environment. While visible contaminants are not tested routinely, their removal is integral to hygiene protocols given the risk that they may present, such as microbial contamination. Fluorescence extends this principle by making previously invisible contamination visible, and therefore despite its non-specific outcome, the presence of fluorescent matter is a clear, objective indicator that contamination remains — whether organic or chemical — and suggests a failure in cleaning practice. It was not surprising to find fluorescent substances in occupied hospital spaces as many substances fluoresce [20]. This was considered an advantage for general cleaning. Traditional wiping techniques may not remove substances that adhere strongly or are pushed into hard-to-reach areas, which is something that visual inspections alone cannot confirm. Whilst chemical disinfection may be necessary for a specific infection outbreak, the basic removal of physical matter as an infection control tool is recognized [21–24], and fluorescence may support this.

Although fluorescence cannot currently determine the origin of detected contamination, the frequent and statistically higher levels of ATP ($P \leq 0.05$) in fluorescent areas confirmed its ability to detect organic material that may contribute to microbial growth. ATP testing is a well-established cleaning monitoring and training method used in research and operational settings [12,25]. Its inclusion in this study served as a benchmark due to its broad ability to detect biological material. While it does not indicate bacterial load specifically and its limitations are acknowledged [13,26], it remains a valuable and accepted proxy for cleanliness assessments, particularly in studies focused on cleaning rather than infection transmission specifically [27], and given the resource-intensive limitations of microbial testing [28]. Microbial contamination is central to infection prevention and control work, but it is not the only contaminant that presents human risk.

Additionally, it is widely established that saliva, urine and other body fluids fluoresce [16,29,30], which pose infection risk [7,31]. Food residues, which frequently fluoresce, may fuel bacterial growth, contribute to biofilms, and present allergenic risk through cross-contamination [32]. Riboflavin (vitamin B2) is naturally present in a variety of foods, including dairy products, eggs, green leafy vegetables, meat, fish, nuts and fortified cereals [33]. It is also found in body fluids such as blood [33], urine [34] and breast milk [35], typically as part of coenzymes such as flavin mononucleotide and flavin adenine dinucleotide. Consequently, its presence as an environmental contaminant would not be unusual. If riboflavin contamination existed as part of food waste, it could theoretically provide a reservoir for bacterial growth or contribute to biofilm production [36].

Riboflavin fluoresces readily under UV or blue light [37], meaning that it is highly possible that riboflavin-containing substances were detected.

In an operational hospital, contamination is likely to compromise a complex mixture of contaminants. For example, a bed head may be touched by the patient, nurses, porters, visitors etc. Each person would add contamination depending on what they have eaten, how they sweat, when they last washed, etc. They may have soap residue, sanitizer, cosmetic products, or food on their hands, much of which is likely to fluoresce. This complexity would be reflected in an FTIR spectrum and require specialist interpretation, although it could be useful in detection of a particular contaminant.

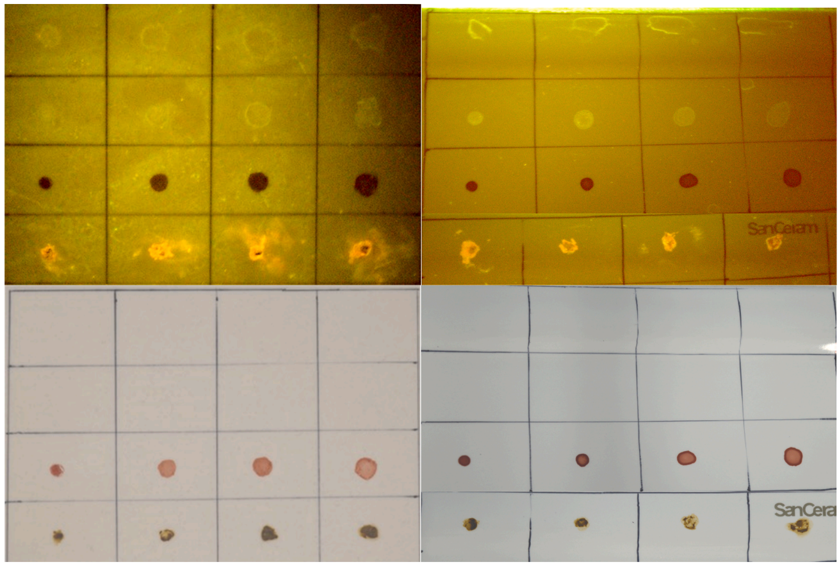


Figure 3. Top row shows 0.5 μL saliva, urine, blood and faeces. Columns (left to right) show neat fluid (approx. 2 mm in diameter), 1:2 dilution in tap water, 1:5 dilution in tap water and 1:10 dilution in tap water. Tap water was used as the dilutant because it is likely to be found in a sink and is the key dilutant in cleaning fluids. The droplets increase in diameter due to viscosity.

An isolated fluorescent sample, examined under equivalent light conditions consecutively, should provide a consistent fluorescent response. The fluorescence intensity may change over time due to molecular changes. However, intra- and interperson variation exists. For instance, if two samples of saliva from the same person or another person were examined under UV/blue light, there may be variation in fluorescence intensity due to natural biological variation and factors such as recent food intake, oral hygiene, hydration status, and health conditions. Figure 3 contains surfaces from a university hospital teaching simulation suite. Both contain two visible contaminants (blood and faecal matter) and two invisible contaminants (saliva and urine).

Blood and faeces may or may not be seen during a visual inspection due to surface contrast, visual constraints, and human error. On light-coloured substrates, at 0.5 μL, urine and saliva droplets are imperceptible until fluoresced. Urine and saliva appear quite different depending on the substrate, yet they originate from the same person/donation. Blood and faeces also differ in appearance, with fluorescent aspects to the faeces which may aid detection. Blood absorbs the incident light, which is why it appears darker. This can be highly advantageous for detection on surfaces that do not absorb light (due to contrast). Consequently, although it is not possible to determine from looking at a fluorescent sample what it actually is, the value in seeing contamination which could be a risk, confirmed in the literature and underpinned by sound scientific principles, led us to suggest that the sensible approach would be to remove fluorescent contamination from a hospital environment through cleaning, and that by doing so, this could provide a safer environment. It is of the same principle that during visual inspections, the nature of the contaminants is unknown, but they are removed for the risk that they may present. This work has been presented as preliminary, but further investigation is encouraged for these reasons.

The detection of alternative substances using fluorescence is advantageous for cleaning because other toxic chemicals, such as cleaning fluids or drug residues, may have fluorescent properties and/or residue in residual contamination. For example, antineoplastic drugs pose significant health risks for healthcare workers who prepare, administer or handle these medications and/or contaminated materials [38,39]. Confirmation of their presence is only possible with chemical analysis, but from a cleaning perspective, removing contamination which may or may not contain toxic chemicals is highly likely to broadly improve the safety of a hospital environment. During this study, the lower sections of the walls were often covered with fluorescent spatter that would logically occur as a floor was mopped. Although further analysis would be necessary to confirm the presence of cleaning fluid and/or any other contaminant, the shape, position and quantity of contamination could be a useful indicator of its type.

The finding that there was no concerning difference in the contamination detected between the blue Class 2 and Class 3[c] light sources was encouraging. This indicated that using a lower-powered Class 2 device did not increase false-negative results, and, as a Class 2 device, it can be used safely in situations where accidental, brief exposure to the eyes is possible but unlikely to cause harm [40]. While a Class 3 source might theoretically offer enhanced sensitivity, any potential advantage must be carefully weighed against the heightened health and safety risks associated with its use. A user should carefully consider the specification of the device because the intensity of the incident light and bandwidth (wavelength spread) will impact the results. For instance, insufficient power will likely increase the false-negative rate (i.e. fail to fluoresce what a more powerful instrument would fluoresce).

Portable light sources can be positioned at a distance and angle to enable optimal fluorescence. This is a useful attribute

[c] Light sources are classified according to the potential risk they pose to human eyes and skin, detailed by the Health and Safety Executive [40].

because the user can utilize the device to their advantage. Training could advise users of compatible surfaces and flexibility in the use of the devices. Alongside training, adherence to a stringent risk assessment to prevent harm to users from high-intensity light exposure may be achieved through ergonomic design (i.e. through the use of a bellow) and the application of devices of the minimum power required to achieve the desired outcome.

Variation in contamination levels across different surfaces was likely influenced by surface design, texture, usage, and ease of access for cleaning. For example, bed frames that were constructed with rounded rails and recessed corners are logically more difficult to clean. Overbed tables, while flat and apparently easy to wipe down, are high-contact surfaces and often textured, allowing contamination to become trapped. These proof-of-concept results are encouraging as the surfaces that were evaluated are representative of other NHS hospital furniture. White ceramic, grey and white plastics, and enamelled metal are typical, suggesting that the method would work in alternative environments. An unsuitable surface could be wiped using a typical hospital cleaning wipe, and the wipe examined for removed fluorescent substances. This expands its potential and adds discretion to the method.

Notably, more fluorescent substances were found on the underside of overbed tables, a part perhaps accessed less frequently during routine cleaning despite its role in repositioning the table for patient use. Fluorescence could help identify locational patterns to direct cleaning processes and assess effectiveness using evidence-based data. This is partly attributed to its ability to screen surface areas, in marked contrast to ATP testing which is often perceived by staff as a guessing game akin to playing 'battleships', even when focused on known high-touch areas. ATP testing is practically limited by cost, time and spatial constraints, with each swab covering just 10 cm^2, whereas fluorescent screening covers broader areas (approximately 40 cm^2) and has no ongoing consumable cost. A threshold of $\leq$50 RLU was used because this was the standard of the collaborating health board. There are no universal benchmarks for ATP testing [26,41], and therefore it is entirely possible that viable microbes were present below this level. If the surface is cleaned after locating fluorescent contamination that contained ATP, it is reasonable to assume that ATP levels will decrease. It is crucial to consider that many substances lack detectable ATP, such as viruses or bacterial spores [32].

The absence of fluorescent substances should not be taken as definitive proof that no contamination is present. This was evidenced in this study by false-negative ATP results. Not all substances fluoresce, and in some cases, the amount of fluorescent material may fall below the detection threshold of the human eye, including contamination that has a high microbial load. However, the method undoubtedly detected significantly more contamination than visual inspection.

The detection of fluorescent contamination using a portable torch is subject to human error as it is the user who interprets what they can see. For example, if a surface is partially fluorescent, a user may be unsure if they are seeing contamination. The simple solution is to wipe the surface and to see if the fluorescent substance is removed. During the study, the difference between the surface and contamination was apparent given the shape and intensity of the contaminant. For instance, drips, handprints, spatter etc. (see Figure 1). Some surfaces absorb light partially or wholly [20] and appear black when these torches are used, which would be clear to a user. Examples in this study were the television and door panels, which were dark green/blue. This can make fluorescent substances difficult to observe unless the fluorescence is particularly intense.

To improve reliability and scalability, the authors' ongoing work includes the development of artificial intelligence (AI)-powered image analysis tools to automate the detection of fluorescent substances to reduce subjectivity. The AI algorithm addresses the variability and potential interference caused by different surface materials in fluorescence detection by training on a diverse dataset of hospital equipment surfaces. Sample images of both clean and unclean surfaces, including those made from a variety of materials commonly found in clinical settings (e.g. bed, chair, sink, mattresses), are collected and labelled. By exposing the model to verified examples of contamination and clean backgrounds (Figure 3), the AI learns to recognize the characteristic fluorescence patterns associated with actual contaminants, while distinguishing them from background signals caused by materials such as adhesives or surface coatings. This approach enables the AI to generalize across heterogeneous environments, and improves the reliability of detection.

This machine learning model will enable quantitative estimates to be made of surface contamination on different surfaces, supporting comparative analysis of different types of light and helping to identify trends in the surface types that have the greatest levels of contamination.

In conclusion, filtered blue and UV light rapidly detected fluorescent contamination that was not visible to the naked eye and was missed during routine hospital cleaning. Fluorescent residues were found on every surface examined, and varied in appearance and frequency depending on the item. In 64% of cases, fluorescent areas had significantly higher ATP levels than adjacent non-fluorescent areas, indicating the presence of organic material as well as alternative contamination. Significant differences were particularly evident on high-touch items such as patient chairs, bed frames, overbed tables, bedside units and pillows.

While not all contamination fluoresces, and surface properties can affect visibility, the high prevalence of fluorescent substances on 'visibly clean' surfaces exposed the limitation of visual inspection alone.

This proof-of-concept research encourages further study of fluorescence as a complementary method to environmental monitoring with other recognized benchmark assessments, such as ATP testing. This is because fluorescence can detect other, potentially harmful substances, such as cleaning residues or drug traces that reside in fluorescent matter. The method is also quicker, covers a larger surface area, and has no ongoing consumable costs. Using this method as part of cleaning routines or audits could help improve cleanliness and, ultimately, support safer environments for patients, staff and carers, but would require investment in equipment and expertise. The authors emphasize that implementation is not encouraged without further study, and that informed, considered judgement is required, particularly given the critical importance of hospital hygiene to human health.

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Conflict of interest statement

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Ethical approval

The Health Research Authority, and Health and Care Research Wales approved this study (IRAS project ID: 318094, REC reference: 22/HCRW/0031).

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