

Evidence-Based Hygiene Verification

Integrating Alternate Light Source (ALS) Inspection and Ultraviolet-C (UVC) Decontamination into a Next-Generation Quality Assurance Framework

Scientific White Paper

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Foreword

Beyond Visual Cleanliness

This white paper originated from the development of a novel, patent-protected portable solution designed to strengthen hygiene safety in public environments. The objective behind the product is straightforward: to provide operators with a practical method to visualize biological residues that remain invisible under normal lighting, decontaminate the identified area, and verify the result within a single, integrated workflow (Thathalo Aldapt technical specification, 2026).

The need for such a system is particularly critical in high-traffic public environments, where large numbers of people repeatedly use shared facilities and touch the same surfaces. The challenge addressed here is not general food safety, which is governed by established controls, but rather the hygiene gap at the interface of people, shared sanitary facilities, high-touch surfaces, and environments where food is served or consumed. A restaurant, service station, airport, shopping centre, hotel, hospital, school, or other busy facility may maintain rigorous operational standards while still facing continuous recontamination of door handles, flush controls, faucets, dispensers, counters, touchscreens, and other shared contact points.

This challenge led directly to the development of the integrated ALS/UVC platform described in this white paper. This patent-protected approach combines functions that are traditionally separated: biological residues are first visualized using Alternate Light Source (ALS) fluorescence inspection; the identified area is then subjected to targeted dual-wavelength Ultraviolet (UVC/UVA) decontamination; and the treated area is subsequently inspected again to verify the outcome. In operational terms, the system establishes a closed-loop process of:

Detect -> Decontaminate

The purpose is to elevate public hygiene safety by making contamination control targeted, visible, repeatable, and verifiable. Instead of relying solely on whether a surface appears visually clean, personnel can accurately pinpoint where biological residue remains, intervene exactly where action is required, and confirm the condition of the surface immediately afterward. This creates a direct, actionable link between detection, corrective action, and verification.

The concept unites established principles of forensic optical inspection with advanced germicidal UV technology in a groundbreaking portable application. While forensic ALS technology has been used for decades to reveal hidden biological traces, and UVC is a proven method for microbial inactivation, the innovation described here lies in merging these complementary capabilities—enhanced by precision optics—into a single practical platform and workflow for evidence-based hygiene verification.

Therefore, this white paper was developed not simply as a general discussion of hygiene technology, but as the scientific and operational foundation for this specific product concept. It examines the underlying optical and microbiological principles, the limitations of conventional visual inspection, the synergistic role of UVC decontamination, observations

from real-world pilot evaluations, and the requirements for systematic scientific validation and practical implementation.

The proposed approach does not replace established cleaning procedures, microbiological testing, or infection-prevention protocols. Rather, it adds a crucial new layer of evidence and intervention: make residual biological contamination visible, decontaminate the identified area, complete the required physical cleaning, verify the result, and document the action.

The broader ambition is to contribute to safer public environments by moving hygiene management from an assumption-based model toward an evidence-based standard. In spaces shared by many, hygiene should no longer be judged merely by what appears clean—it should increasingly be supported by what can be detected, treated, verified, and documented.

Executive Summary

Executive Summary

Beyond Visual Cleanliness

Routine cleaning is essential in healthcare, food service, hospitality, public transportation, and other high-traffic environments. However, visual inspection alone cannot reliably confirm whether biological or organic residues have been effectively removed from high-touch surfaces.

This white paper presents an Evidence-Based Hygiene Verification framework that combines fluorescence-assisted inspection, targeted dual-wavelength (UVC/UVA) decontamination, physical cleaning, post-cleaning verification, and digital documentation into a single, closed-loop workflow:

Detect → Decontaminate

Alternate Light Source (ALS) fluorescence inspection rapidly reveals residual biological or organic material that remains invisible under normal lighting. It does not identify microorganisms, determine viability, or confirm specific pathogens; therefore, ATP testing and microbiological sampling remain necessary where quantitative hygiene or microbial assessment is required.

The workflow is specifically designed to treat identified hotspots with targeted UV decontamination *before* physical contact. Conventional physical cleaning then removes the remaining organic material, ALS reinspection verifies the visual result, and a digital application documents the completed intervention.

A qualitative pilot evaluation in publicly accessible restrooms demonstrated that fluorescence inspection successfully detected localized residues on surfaces that appeared visually clean, particularly on high-touch points and concealed structural areas. Repeated inspection and corrective cleaning progressively reduced observable hotspots, illustrating the immense value of immediate visual feedback. Note that the pilot did not include ATP measurements, microbiological cultures, or quantitative microbial-reduction testing.

To support routine point-of-use application, the white paper outlines a patent-protected handheld platform that integrates ALS detection and high-intensity dual-wavelength decontamination into a compact, battery-powered device weighing approximately 290 g.

Crucially, the platform introduces a powerful biological synergy by combining 265 nm UVC with 365 nm UVA. This simultaneous dual-exposure prevents pathogen photoreactivation. While the 265 nm UVC directly damages the pathogen's DNA, the 365 nm UVA wavelength generates a localized surge of Reactive Oxygen Species (ROS) that destroy DNA repair enzymes. This secondary oxidative strike renders the UVC-induced genomic damage completely irreversible.

To deliver this synergy effectively, the hardware platform is specified to provide:

- **Precision-engineered dual 5° collimated optics** delivering simultaneous 265 nm UVC (35 mW/cm²) and 365 nm UVA (1.5 W) perfectly co-aligned at a 10 cm working distance.
- **An Orange Halo Distance Guide** for visually confirming repeatable positioning.
- **A gyroscope-controlled automatic safety switch** to prevent accidental operator exposure.
- **App-supported digital documentation** for traceable quality assurance.

Chapter 1

Introduction

Beyond Visual Cleanliness

For decades, hygiene management has focused primarily on ensuring that facilities appear clean. Across hospitals, hotels, airports, restaurants, schools, commercial buildings, and public infrastructure, cleaning quality has traditionally been assessed through visual inspection, standardized procedures, and routine supervisory audits. While these methods remain essential for operational control, they provide only limited evidence that cleaning has effectively removed residual biological contamination.

Advances in microbiology, infection prevention, and environmental hygiene have demonstrated that visual cleanliness is not synonymous with hygienic cleanliness. Surfaces appear spotless while retaining invisible organic residues such as proteins, skin secretions, body fluids, or other biological material. Although these residues do not necessarily indicate the presence of pathogenic microorganisms, they indicate incomplete cleaning and identify areas where hygiene performance can be improved (Dancer, 2009; Otter et al., 2013; Weber et al., 2013).

The COVID-19 pandemic further emphasized the importance of objective hygiene verification. Organizations across healthcare, hospitality, transportation, food service, and public services are increasingly expected not only to perform cleaning, but also to demonstrate its effectiveness. Consequently, hygiene management has evolved from a routine operational activity into a critical component of quality assurance, regulatory compliance, customer confidence, and organizational resilience.

Existing verification methods each address only part of this challenge. Visual inspections assess appearance, cleaning checklists confirm process compliance, ATP measurements quantify residual organic material within limited sampling areas, and microbiological cultures identify viable microorganisms but require laboratory analysis and delayed results. While each method provides valuable information, none alone offers rapid, non-contact, large-area verification of residual biological contamination during routine operations (Boyce, 2016; Nante et al., 2017).

Recent advances in optical technology provide an opportunity to bridge this gap. Alternate Light Source (ALS) systems enable the visualization of naturally fluorescent biological residues that remain invisible under conventional illumination. When combined with targeted Ultraviolet-C (UVC) decontamination, these technologies establish an integrated quality assurance process that supports objective inspection, evidence-based intervention, and immediate verification.

Chapter 2

The Hidden Reality of Biological Contamination

Why "Clean" Does Not Always Mean Hygienic

The visual appearance of a surface is often regarded as an indicator of hygiene. In reality, however, visual cleanliness and hygienic cleanliness represent fundamentally different concepts. While visible dirt, dust, and stains can usually be detected by the human eye, microscopic biological residues frequently remain unnoticed despite routine cleaning.

These residues include proteins, skin secretions, perspiration, saliva, urine, blood, food residues, and other organic material. Although their presence does not necessarily indicate pathogenic microorganisms, they provide objective evidence that biological material remains on a surface and indicate incomplete cleaning.

For hygiene management, this distinction is essential. The objective is not merely to achieve visually clean environments but to minimize residual biological contamination that contributes to microbial persistence and cross-contamination.

2.1 High-Touch Surfaces: Primary Reservoirs of Residual Contamination

Biological residues do not accumulate uniformly throughout an environment. Instead, contamination is concentrated on surfaces that are repeatedly touched during normal human activity (Dancer, 2009; Weber et al., 2013).

Typical high-touch surfaces include:

- Door handles
- Flush buttons
- Faucet controls
- Soap dispensers
- Paper towel dispensers
- Handrails
- Elevator buttons
- Payment terminals
- Touch screens
- Waste-bin lids

Each contact transfers microscopic quantities of skin cells, proteins, lipids, perspiration, and microorganisms. Over time these deposits accumulate, creating predictable contamination hotspots that deserve particular attention during cleaning and verification (Kramer et al., 2006; Otter et al., 2013).

Understanding these behavioral contact patterns allows cleaning resources to be focused where they provide the greatest benefit.

2.2 Organic Residues as Indicators of Cleaning Performance

Organic residues should not be interpreted as evidence of infection. Instead, they serve as objective indicators of cleaning effectiveness.

Many biological materials contain naturally fluorescent compounds that become visible under appropriately selected illumination wavelengths. Their detection therefore provides rapid visual feedback regarding the presence of residual biological material without requiring laboratory analysis.

Within the framework proposed in this white paper, fluorescence is used as a quality assurance indicator. It identifies surfaces where cleaning performance requires improvement and supports targeted corrective action.

2.3 Biofilms: A Persistent Hygiene Challenge

Biofilms represent one of the most significant challenges in environmental hygiene. They consist of structured microbial communities enclosed within a self-produced extracellular matrix that adheres strongly to natural and artificial surfaces (Flemming & Wingender, 2010).

Once established, biofilms exhibit increased resistance to mechanical cleaning and chemical disinfectants, making them difficult to remove (Flemming & Wingender, 2010).

Although ALS technology is not designed to detect biofilms directly, repeated fluorescence observed at the same locations identifies areas where residual organic material persists and where biofilm formation is more likely. Such findings can guide targeted investigation and preventive maintenance.

2.4 Cross-Contamination

Every interaction between people and their environment creates opportunities for contamination transfer.

Residual biological material deposited on frequently touched surfaces is transferred to hands and subsequently to additional surfaces, equipment, or objects. This process, known as cross-contamination, is particularly relevant in environments with high visitor turnover and numerous shared contact points.

Reducing contamination at these critical interfaces is therefore an important objective of evidence-based hygiene management.

2.5 The Human Factor

Cleaning quality depends not only on procedures but also on human performance. Time constraints, workload, staff turnover, and varying levels of experience all influence cleaning outcomes.

Objective visual feedback enables cleaning personnel to identify overlooked areas immediately and adjust their techniques accordingly. Rather than serving solely as an inspection method, fluorescence-assisted verification also functions as an effective training and continuous improvement tool.

2.6 From Assumption to Evidence

Traditional hygiene inspections rely largely on visual assessment. While suitable for evaluating appearance, they provide only limited information about residual biological contamination.

Objective optical inspection complements conventional cleaning verification by revealing biological residues that would otherwise remain undetected. This enables organizations to shift from assumption-based evaluations toward measurable, evidence-based hygiene verification.

The following chapter reviews current hygiene verification methods and examines how fluorescence-assisted inspection complements established techniques such as ATP testing and microbiological analysis.

Chapter 3

Current Methods of Hygiene Verification

Strengths, Limitations and the Need for Objective Assessment

Reliable hygiene management requires not only effective cleaning procedures but also robust methods to verify their outcome. Across healthcare, hospitality, food service, transportation, and commercial facilities, several complementary approaches are currently used to assess hygiene performance. Each provides valuable information but addresses a different aspect of cleanliness. Understanding their respective strengths and limitations highlights the need for a more comprehensive verification strategy.

3.1 Visual Inspection

Visual inspection remains the most widely used hygiene verification method because it is rapid, inexpensive, and easy to implement. It effectively identifies visible dirt, stains, dust, and general housekeeping deficiencies (Boyce, 2016).

However, visual assessment evaluates appearance rather than residual biological contamination. Invisible organic residues cannot be detected under conventional lighting, and inspection results vary between observers. Consequently, visual inspection alone provides only limited assurance of cleaning effectiveness.

Strengths

- Rapid and inexpensive
- No specialized equipment required
- Suitable for routine inspections
- Easy to standardize

Limitations

- Detects only visible contamination
- Subjective evaluation
- Cannot identify residual biological material

3.2 Cleaning Checklists and Standard Operating Procedures

Cleaning schedules and Standard Operating Procedures (SOPs) ensure that cleaning activities are performed consistently and according to defined processes. They remain fundamental components of quality management and regulatory compliance.

Nevertheless, completed checklists verify that prescribed tasks have been performed—not whether cleaning has successfully removed biological contamination. Process compliance should therefore not be mistaken for outcome verification.

3.3 ATP Bioluminescence Testing

ATP bioluminescence provides a rapid, quantitative assessment of residual organic material by measuring adenosine triphosphate (ATP) on sampled surfaces. It is widely used in healthcare, food production, pharmaceutical manufacturing, and hospitality (Nante et al., 2017; Sanna et al., 2018).

Although ATP testing offers objective numerical results, it evaluates only small sampling areas, requires consumable swabs, and does not distinguish between harmless and hazardous organic material. As a result, large facilities require extensive sampling to obtain representative coverage (Omidbakhsh et al., 2014; Nante et al., 2017).

Strengths

- Rapid quantitative results
- Objective measurements
- Established methodology
- Suitable for trend analysis

Limitations

- Limited sampling area
 - Ongoing consumable costs
 - No visual overview of contamination
 - Does not identify microorganisms
-

3.4 Microbiological Surface Sampling

Microbiological culture remains the reference method for identifying viable microorganisms. It provides highly reliable information regarding microbial species and contamination levels and is indispensable during outbreak investigations and scientific validation (Rutala & Weber, 2019).

Its routine use for operational hygiene monitoring is limited by laboratory requirements, delayed results, and selective sampling. Consequently, microbiological analysis is best suited for confirmation rather than rapid day-to-day quality assurance.

Strengths

- High scientific accuracy
- Identification of microorganisms
- Accepted by regulatory authorities

Limitations

- Slow turnaround
- Laboratory dependent
- High operational costs
- Limited sampling coverage

3.5 Fluorescent Marker Systems

Fluorescent marker systems evaluate cleaning performance by applying invisible tracers to selected surfaces before cleaning. Successful removal of the marker indicates that the surface has been cleaned.

These systems are valuable training tools but assess only the removal of an artificial marker rather than naturally occurring biological residues. Their routine use also requires preparation before every inspection.

3.6 Alternate Light Source (ALS) Fluorescence Inspection

Recent advances in forensic optical technology have introduced a different approach to hygiene verification. Instead of analysing small samples or applying artificial tracers, ALS systems visualize naturally fluorescent biological residues across large surface areas within seconds.

ALS does not identify microorganisms or quantify infection risk. Rather, it provides objective visual evidence of residual biological material, allowing inspectors to rapidly identify contamination hotspots and focus corrective actions where they are most needed (Croce & Bottiroli, 2014).

3.7 Comparative Overview

Method	Detects Organic Residues	Identifies Microorganisms	Large Area Coverage	Immediate Results	Consumables
Visual Inspection	X	X	✓	✓	X
Cleaning SOPs	X	X	✓	✓	X
ATP Testing	✓	X	X	✓	✓
Microbiological Culture	Indirect	✓	X	X	✓
Fluorescent Markers	Artificial Marker Only	X	Limited	✓	✓
ALS Fluorescence	✓	X	✓	✓	X

3.8 Towards Evidence-Based Hygiene Verification

No single verification method can fully assess environmental hygiene.

Visual inspection evaluates appearance. ATP testing quantifies residual organic material within sampled areas. Microbiological analysis identifies viable microorganisms. Fluorescent markers assess cleaning performance using artificial tracers.

ALS complements these established approaches by providing rapid, non-contact visualization of naturally occurring biological residues across large surface areas. Rather than replacing existing methods, it fills the gap between subjective inspection and laboratory-based analysis, enabling organizations to move toward evidence-based hygiene verification.

The next chapter explains the scientific principles that make fluorescence-assisted inspection possible and examines why ALS has become an established tool in forensic science and an emerging technology in environmental hygiene.

Chapter 4

The Science Behind Alternate Light Source (ALS) Technology

Making the Invisible Visible

The human eye is highly effective at detecting visible dirt and surface defects but is limited to a narrow range of the electromagnetic spectrum. As a result, many biological residues remain invisible under conventional white light despite being present on a surface.

Alternate Light Source (ALS) technology overcomes this limitation by illuminating surfaces with selected wavelengths that interact differently with biological materials. Through fluorescence, absorption, and contrast enhancement, ALS enables the visualization of organic residues that cannot be detected during routine visual inspection.

Originally developed for forensic science, ALS has become an established tool for biological evidence detection, document examination, trace analysis, and industrial inspection. The same optical principles also provide significant potential for objective hygiene verification.

4.1 What Is an Alternate Light Source?

An Alternate Light Source is a controlled illumination system capable of emitting narrow spectral bands across the ultraviolet, visible, and near-infrared regions of the electromagnetic spectrum.

Unlike white light, which contains a broad mixture of wavelengths, ALS selectively excites optical properties within different materials. Depending on the wavelength used, biological residues fluoresce, absorb light, or exhibit enhanced contrast against the surrounding surface.

Typical forensic ALS systems operate within the following spectral regions:

Spectral Region	Approximate Wavelength
------------------------	-------------------------------

Ultraviolet	365–400 nm
Violet	405–430 nm
Blue	430–500 nm
Green	500–570 nm
Yellow	570–590 nm
Orange	590–620 nm
Red	620–700 nm
Near Infrared	700–1000 nm

4.2 The Principle of Fluorescence

Fluorescence occurs when molecules absorb photons of one wavelength and almost immediately emit light at a longer wavelength (Lakowicz, 2023; Valeur & Berberan-Santos, 2012; see Appendix F).

The process involves four fundamental steps:

1. Excitation by incident light.
2. Transition to a higher energy state.
3. Partial energy loss through non-radiative relaxation.
4. Emission of fluorescence at a longer wavelength.

Because biological materials contain different naturally fluorescent compounds, each produces characteristic fluorescence patterns under ALS illumination (Ramanujam, 2000; Croce & Bottioli, 2014; see Figure F-3 in Appendix F).

4.3 Biological Autofluorescence

Biological fluorescence results from the combined emission of several naturally occurring fluorophores rather than a single molecule (Ramanujam, 2000; Croce & Bottioli, 2014; see Appendix F).

Major contributors include:

Aromatic amino acids

- Tryptophan
- Tyrosine
- Phenylalanine

Structural proteins

- Collagen
- Elastin
- Keratin

Metabolic cofactors

- NADH
- NADPH
- FAD
- FMN

Other endogenous fluorophores

- Porphyrins
- Lipofuscin

- Bilirubin (under specific conditions)
- Oxidized lipid products

The fluorescence observed during ALS inspection therefore reflects the combined optical response of multiple endogenous compounds. Fluorescence intensity depends on factors including excitation wavelength, residue composition, hydration, substrate material, residue age, and optical filter selection (Croce & Bottiroli, 2014; Richards-Kortum & Sevick-Muraca, 1996).

Accordingly, ALS should be regarded as a **screening technology for residual biological material**, not as a method for biochemical identification.

4.4 Reflection, Absorption and Contrast Enhancement

Not all observations during ALS inspection result from fluorescence.

Differences in surface reflection, wavelength-specific absorption, and light scattering also contribute to image contrast. Many biological deposits become visible because they alter these optical properties relative to the surrounding material.

Modern ALS inspection therefore combines fluorescence with multiple complementary optical mechanisms to maximize detection sensitivity.

4.5 Optical Filters

Barrier filters are essential components of every ALS system (Lakowicz, 2023; ASTM E1412).

Their function is to suppress reflected excitation light while transmitting emitted fluorescence, thereby increasing image contrast and enabling weak fluorescence signals to be observed (Lakowicz, 2023; Valeur & Berberan-Santos, 2012).

Common barrier filters include:

- Yellow
- Orange (used in our Pilot Study and Product)
- Red

Appropriate filter selection is critical for reliable fluorescence visualization

4.6 ALS in Forensic Science

ALS has been an integral part of forensic investigations for decades.

Typical applications include:

- Biological evidence detection
- Fingerprint enhancement
- Document examination
- Erased or altered writing
- Bruise visualization
- Fibre examination
- Paint comparison
- Trace evidence analysis

Its non-contact, non-destructive nature and immediate results have made ALS a standard tool in forensic laboratories worldwide.

4.7 ALS in Hygiene Verification

The application of ALS to hygiene verification represents a logical extension of established forensic methodology.

Instead of detecting forensic evidence, ALS identifies residual biological material following routine cleaning. Large surface areas can be inspected within minutes, enabling rapid identification of contamination hotspots and supporting quality assurance, staff training, facility audits, and contractor evaluation.

4.8 Strengths and Limitations

Strengths

- Immediate results
- Non-contact inspection
- Large-area assessment
- No consumables
- Repeatable observations
- Permanent photographic documentation
- Effective training and audit support

Limitations

ALS does not:

- identify bacterial species;
- identify viruses;
- quantify microbial load;
- determine infection risk;
- replace microbiological culture;
- replace ATP measurements where quantitative residue analysis is required.

Instead, ALS should be regarded as a complementary inspection technology that identifies where additional cleaning or investigation is required.

4.9 Conclusion

Alternate Light Source technology extends the capabilities of conventional hygiene inspection by revealing biological residues that remain invisible under standard illumination. Rather than replacing established microbiological or biochemical methods, ALS provides a rapid, objective, and non-destructive means of visualizing residual organic contamination across large surface areas.

By bridging the gap between subjective visual inspection and laboratory-based analysis, ALS establishes the optical foundation for evidence-based hygiene verification. Combined with UVC decontamination, discussed in the following chapter, it forms the first component of an integrated quality assurance framework.

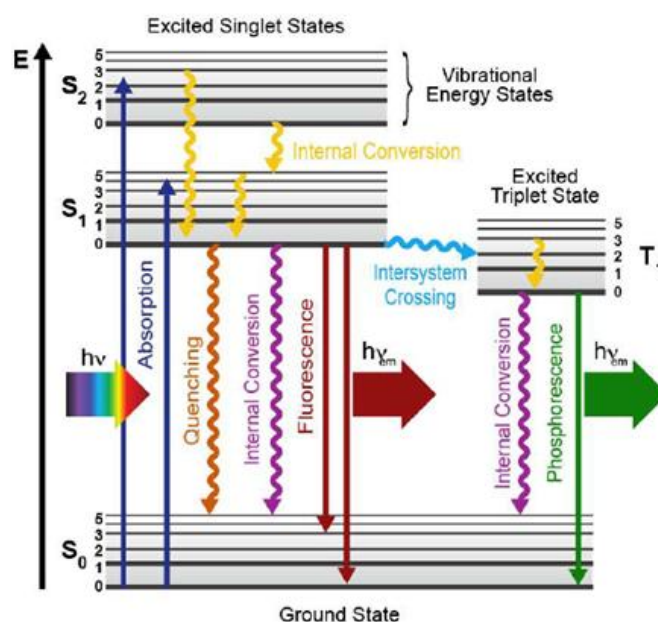


Figure 1. Jablonski energy diagram of electronic and vibrational energy levels of an excited molecule.

Figure 1. Jablonski diagram illustrating the photophysical processes underlying fluorescence. After excitation by an Alternate Light Source (ALS), endogenous fluorophores absorb photons and transition to an excited electronic state. Following rapid vibrational relaxation and internal conversion, the molecules return to the ground state by emitting fluorescence at a longer wavelength (Stokes shift). This wavelength shift enables emitted fluorescence to be separated from excitation light using optical barrier filters, providing the basis for fluorescence-assisted detection of residual biological material.

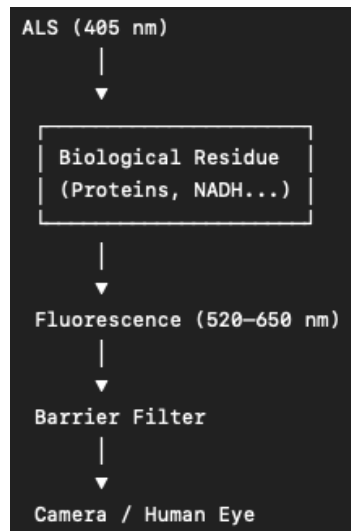


Figure 2. Normalized excitation/absorption and fluorescence emission spectra of representative endogenous biological fluorophores. Dashed curves indicate absorption or excitation characteristics, while solid curves represent fluorescence emission. Vertical markers identify the ALS illumination wavelengths available in the inspection system. Spectral positions are approximate and may vary according to molecular binding, pH, residue age, concentration, and substrate conditions. Adapted from Islam et al. (2013), licensed under CC BY 3.0.

Chapter 5

Ultraviolet-C (UVC) Decontamination

From Detection to Targeted Decontamination

Alternate Light Source technology enables the visualization of residual biological material but does not remove contamination or inactivate microorganisms. Once areas requiring corrective action have been identified, an effective decontamination method is needed.

Ultraviolet-C irradiation is a well-established, non-chemical technology used for the disinfection of water, air, equipment, and environmental surfaces. When correctly applied, UVC rapidly reduces microbial contamination without leaving chemical residues. Its effectiveness, however, depends on the delivered dose, surface condition, exposure geometry, and the susceptibility of the target organism (Kowalski, 2009; CDC, 2023a).

Within the proposed framework, UVC complements cleaning and ALS inspection by providing a targeted intervention following the detection of residual contamination.

5.1 Ultraviolet-C Radiation

Ultraviolet radiation occupies the region of the electromagnetic spectrum between visible light and X-rays. It is commonly divided into three bands:

UV region	Wavelength	Principal characteristics
UVA	315–400 nm	Fluorescence excitation, photochemical effects and skin tanning
UVB	280–315 nm	Biological tissue damage and sunburn
UVC	200–280 nm	Strong germicidal activity through nucleic-acid absorption

UVC radiation from the sun is largely absorbed by the atmosphere and is therefore generated artificially for disinfection.

Common germicidal sources include:

- Low-pressure mercury lamps, typically emitting near 254 nm
- Excimer lamps, including Far-UVC sources near 222 nm
- UVC LEDs, commonly operating between approximately 260 and 280 nm

Wavelengths near the absorption maximum of nucleic acids are particularly effective for microbial inactivation (Beck et al., 2015; CDC, 2023a).

5.2 Mechanism of Microbial Inactivation

DNA and RNA absorb UVC photons. The absorbed energy induces photochemical lesions, particularly cyclobutane pyrimidine dimers and related photoproducts (Kowalski, 2009; CDC, 2023a).

These lesions interfere with:

- DNA replication
- RNA transcription
- Protein synthesis
- Cell division
- Viral replication

Microorganisms exposed to a sufficient UVC dose lose their ability to reproduce and are therefore considered inactivated (Kowalski, 2009; Bolton & Cotton, 2011).

UVC does not physically remove microorganisms or organic material from a surface. Cleaning remains necessary to eliminate soiling and improve radiation access to the microbial target.

5.3 Factors Influencing UVC Effectiveness

The performance of UVC decontamination is governed by several interacting parameters.

UVC dose

The delivered dose, or fluence, is typically expressed in millijoules per square centimetre: (Bolton & Cotton, 2011; see Appendix K).

$$\text{Dose} = \text{Irradiance} \times \text{Exposure Time}$$

where irradiance is measured in milliwatts per square centimetre and exposure time in seconds.

The developed handheld platform delivers an irradiance of 35 mW/cm² at a defined working distance of 10 cm. Because delivered dose is the product of irradiance and exposure time, this high irradiance allows the required dose to be achieved within a substantially shorter exposure period. The result is rapid, targeted decontamination that is practical for routine use and immediate intervention during normal operating hours (Thathalo Aidapt technical specification, 2026; microbiological performance and treatment-time claims require validation under Appendix L).

Greater doses generally produce greater microbial reductions, although dose requirements differ substantially among organisms (Bolton & Cotton, 2011; Beck et al., 2015; see Appendix K).

Distance and positioning

Irradiance decreases as the distance from the source increases. Reproducible decontamination therefore requires controlled positioning and a defined working distance (Kowalski, 2009; Feng et al., 2021).

To make this critical parameter easy to control in daily operation, the handheld platform projects an orange Distance Guide onto the treatment surface. When the orange ring appears sharply focused, the correct working distance has been reached. This provides the operator with immediate visual confirmation and supports consistent irradiance, dose delivery, and treatment time without requiring a separate measuring device (Thathalo AIdapt, technical specification, 2026; functional, safety, distance and usability performance require validation under Appendices G and L).

Exposure time

Longer exposure increases the accumulated dose. Portable systems must balance treatment time with the required level of microbial reduction.

Surface geometry

Irregular, porous, recessed, or shadowed surfaces receive an insufficient dose. Direct line-of-sight exposure is therefore an important limitation of conventional UVC systems (Feng et al., 2021; CDC, 2023a).

ThatHalo AIDAPT reaches difficult angles, corners and high-touch areas to deliver a targeted UVC dose directly where it is needed. Its handheld design enables precise positioning, no shadowing and supports effective decontamination of surfaces.

Organic soiling

Proteins, body fluids, dirt, and other organic residues can absorb or scatter UV radiation and shield microorganisms. In the integrated operational workflow, identified hotspots are first subjected to targeted dual-wavelength (UVC/UVA) decontamination before they are touched, reducing the opportunity for viable microorganisms to be transferred through cloths, gloves, hands, or cleaning equipment. Physical cleaning then removes the treated organic residue. Where heavy or opaque soiling prevents adequate UV penetration, gross contamination must first be removed using appropriate protective procedures, followed by the dual-wavelength treatment and final cleaning and verification (CDC, 2023a; Kowalski, 2009).

This point is central to the integrated ALS and dual-wavelength concept: ALS identifies the precise location and extent of residual organic material, allowing the operator to apply the synergistic UVC/UVA treatment directly before physical cleaning while recognising situations in which heavy soiling requires a modified, risk-assessed procedure.

5.3.1 The Dual-Wavelength Biological Synergy (tRNA Degradation)

While standard UV-C radiation effectively inactivates pathogens through direct DNA photolysis, combining it with UV-A (365 nm) creates a highly powerful synergistic effect.

This synergy is driven by a specific molecular vulnerability within the pathogen. Sublethal doses of UV-A light are absorbed directly by 4-thiouridine (s4U) within the pathogen's transfer RNA (tRNA). This UV-A absorption physically crosslinks the tRNA, which immediately reduces the pathogen's amino acid charging capacity and shuts down cellular protein synthesis.

This targeted shutdown creates a massive biological disadvantage for the pathogen. Because the cell's ability to synthesize new proteins is halted, it experiences immediate growth arrest and becomes highly susceptible to UV-C genomic damage. Furthermore, because the pathogen cannot produce the proteins necessary to execute repairs, the subsequent UV-C damage becomes irreversible.

Crucially, this synergistic effect is strongest when UV-C is applied immediately after or simultaneously with the UV-A exposure, which permanently impairs the pathogen's ability to recover up to 24 hours later.

5.4 Logarithmic Reduction

Microbial inactivation is commonly expressed as a logarithmic reduction: (Bolton & Cotton, 2011; see Appendix K).

Log reduction	Microorganisms remaining	Reduction
1-log	10%	90%
2-log	1%	99%
3-log	0.1%	99.9%
4-log	0.01%	99.99%
5-log	0.001%	99.999%
6-log	0.0001%	99.9999%

The required reduction depends on the intended application, target organism, exposure conditions, and relevant standards. A log-reduction claim should therefore always be linked to a validated dose and test protocol.

In many decontamination contexts, 3-log to 4-log reductions are used as practical performance benchmarks, although the required target depends on the organism, application, surface, and applicable validation standard. Appendix K provides representative literature

ranges for UVC dose requirements and illustrates why organism-specific validation is essential.

5.5 Evidence of Germicidal Effectiveness

UVC has demonstrated activity against a broad range of microorganisms, including: (Kowalski, 2009; Rutala & Weber, 2019; CDC, 2023a).

Bacteria

- *Staphylococcus aureus*
- Methicillin-resistant *S. aureus*
- *Enterococcus faecium*
- *Escherichia coli*
- *Salmonella enterica*
- *Pseudomonas aeruginosa*
- *Klebsiella pneumoniae*
- *Clostridioides difficile* spores (higher doses required)

Viruses

- Influenza viruses
- Coronaviruses
- Respiratory syncytial virus
- Adenoviruses
- Rotavirus
- Norovirus surrogates

Fungi

- *Candida albicans*
- *Aspergillus* species

The required dose varies according to organism, strain, surface, environmental conditions, and experimental method. General claims of microbial reduction should therefore not be presented independently of the validated operating conditions (Beck et al., 2015; Bolton & Cotton, 2011; see Appendices K and L).

Spore-forming organisms such as *Clostridioides difficile* are particularly important when comparing UVC performance because bacterial spores generally require substantially higher delivered doses than many vegetative bacteria and enveloped viruses. This makes *C. difficile* a useful challenge organism when evaluating high-intensity UVC systems, provided claims are tied to a defined wavelength, irradiance, exposure time, surface, and validated test protocol (CDC, 2023a; Rutala & Weber, 2019; see Appendices K and L).

5.6 Applied Case Example: *Clostridioides difficile*

Clostridioides difficile provides a relevant challenge example for evaluating targeted dual-wavelength decontamination. It is a spore-forming bacterium associated particularly with healthcare environments. Its spores can survive for extended periods on environmental surfaces and are substantially more resistant than vegetative bacteria and enveloped viruses.

Alcohol-based disinfectants, including isopropanol-based products, do not reliably inactivate *C. difficile* spores. Many routine cleaning and disinfection products are also insufficient unless they have demonstrated sporicidal activity. Effective chemical control therefore requires an approved sporicidal product, the correct concentration, and the specified contact time. Dual-wavelength UV irradiation is not the only method capable of inactivating *C. difficile* spores, but it provides a rapid, non-chemical supplementary intervention when the synergistic dose is delivered directly to the contaminated surface.

Conventional wiping with a non-sporicidal product can remove part of the contamination without reliably inactivating the remaining spores. Cleaning cloths, wipes, gloves, and hands may consequently pick up viable spores and transfer them to adjacent surfaces or other rooms. Experimental studies have demonstrated that contaminated cleaning materials can redistribute *C. difficile* spores across consecutively wiped surfaces. Rather than eliminating the contamination, an inadequately controlled wiping process can therefore enlarge the contaminated area and contribute to cross-contamination.

The proposed AIDAPT workflow addresses this risk by treating a detected hotspot with targeted dual-wavelength (UVC/UVA) radiation before the contaminated surface is physically touched.

At the specified treatment-surface delivery of **35 mW/cm² (265 nm UVC) combined with 1.5 W (365 nm UVA) at 10 cm**, a three-second exposure delivers a highly concentrated incident UVC dose of **105 mJ/cm²** under stable, direct, and unobstructed conditions. For highly resistant organisms like *C. difficile*, the addition of 365 nm UVA provides a critical biological synergy: the UVA-induced Reactive Oxygen Species (ROS) degrade the spore's defensive proteins and repair enzymes, ensuring the UVC-induced genomic damage is irreversible. This dual-action approach overcomes the pathogen's natural resistance faster than single-wavelength systems. However, any specific claim regarding the inactivation or log reduction of *C. difficile* spores must be confirmed through organism-, surface-, and device-specific microbiological validation according to Appendix L.

Conventional room-scale UVC systems can reduce environmental *C. difficile* contamination, but they are generally used as supplementary terminal-room technologies. Their size, positioning requirements, and dependence on direct line-of-sight exposure can limit access to recessed, angled, or shadowed surfaces. AIDAPT's compact handheld design allows the operator to direct the collimated 5-degree dual-wavelength beam towards corners, edges, undersides, and individual high-touch surfaces. Repositioning the device reduces geometric shadowing and enables targeted treatment of areas that may receive an insufficient dose from a stationary room-scale source.

Projected Impact on Treatment Time: A Synergistic Extrapolation

While specific log-reduction data for *C. difficile* under dual-wavelength exposure is currently undergoing formal validation (see Appendix L), the anticipated reduction in treatment time

can be extrapolated from established enzymatic synergy models. Recent studies have demonstrated that adding high-intensity UVA to a UVC source reduces the total dose required to inactivate highly resilient biological proteins by approximately 75%—an effective 4-fold increase in efficiency.

Applying this synergistic multiplier to *C. difficile* yields a compelling operational advantage. Standard UVC alone requires a target dose of approximately 150 mJ/cm² to achieve a >99% reduction in *C. difficile* spores. At the platform's specified UVC irradiance of 35 mW/cm², achieving this baseline dose using UVC alone would take approximately 4.3 seconds.

However, by simultaneously delivering 1.5 W of 365 nm UVA to actively degrade the spore's protective proteins and repair mechanisms, the required UVC target dose is projected to drop to roughly 37.5 mJ/cm². Consequently, the required exposure time to achieve the same >99% reduction could theoretically be reduced to just over 1.1 seconds. Pending final microbiological validation, this drastic reduction in treatment time translates to an unprecedented operational speed for targeted, point-of-use decontamination

After the dual-wavelength treatment, physical cleaning removes the inactivated microorganisms and remaining organic material. ALS reinspection then verifies whether fluorescent residue remains, while digital documentation records the completed intervention. The example illustrates the operational value of combining localized pre-contact dual-wavelength treatment with subsequent removal and verification rather than relying on conventional wiping alone.

Parameter	Standard UVC (Without Synergy)	Dual-Wavelength (UVC + UVA)
Required Target Dose	150 mJ/cm ²	~37.5 mJ/cm ² (<i>extrapolated</i>)
UVC Irradiance (Thathalo)	35 mW/cm ²	35 mW/cm ² (+ 1.5 W UVA)
Required Exposure Time	~4.3 seconds	~1.1 seconds

5.7 Operational Applications

UVC is used as a supplementary decontamination method in:

- Patient rooms
- Intensive care units
- Operating theatres
- Isolation rooms
- Laboratories
- Pharmaceutical and food-production environments
- Public transportation
- Airports
- Hotels
- Schools
- Commercial facilities

Healthcare currently provides the most extensive evidence base for environmental UVC use. Conventional room-scale or terminal UVC is typically deployed after manual cleaning to reduce residual microbial contamination on exposed surfaces. This established sequence differs from the proposed localized pre-contact workflow in this white paper, in which a detected hotspot receives targeted UVC before it is physically touched. The two applications should not be treated as interchangeable: terminal UVC supplements completed cleaning, whereas the proposed pre-contact step is intended to reduce viable microorganisms before subsequent physical removal and requires workflow-specific validation (Kowalski, 2009; Rutala & Weber, 2019; see Appendix L).

The same principle applies to public environments: UVC should strengthen an established cleaning process rather than compensate for inadequate cleaning.

5.8 Safety Considerations

Conventional UVC radiation causes acute injury to the skin and eyes. Direct exposure must therefore be prevented (ISO 15858:2016; CDC/NIOSH, 2024; see Appendix G).

The integrated handheld platform incorporates a gyroscope-controlled safety switch. The system continuously monitors the orientation of the device and automatically interrupts the UVC output when it is raised or pointed towards the operator. This built-in protection helps prevent accidental exposure of the user's eyes and skin during handheld operation (Thathalo AIadapt, technical specification, 2026; functional, safety, distance and usability performance require validation under Appendices G and L).

Far-UVC around 222 nm is being investigated because of its limited penetration into superficial biological tissues. However, safe deployment depends on spectral purity, exposure limits, product design, and applicable regulation. It should not be described as inherently safe without qualification and validated exposure control (Welch et al., 2022; ISO 15858:2016).

5.9 Integration with ALS Inspection

The value of the proposed platform lies in combining complementary functions:

1. **ALS inspection** identifies residual biological material and contamination hotspots.
2. Targeted UVC decontamination reduces viable microorganisms on directly exposed surfaces before physical contact with the hotspot.
3. Physical cleaning removes the treated visible and organic contamination.
4. Verification with ALS confirms whether residual fluorescent material remains and whether further action is required.
5. Digital documentation records the finding, intervention, and verified outcome.

5.10 Conclusion

While single-wavelength UVC is a scientifically established method for microbial inactivation, combining it with 365 nm UVA creates a powerful dual-wavelength synergy that prevents pathogen recovery and accelerates cell death. However, the performance of any UV intervention inherently depends on controlled dose delivery—which is maximized in this platform through precision 5-degree optics—direct exposure, surface condition, and appropriate safety measures.

Its role depends on the application. In conventional terminal decontamination, cleaning removes organic material before supplementary UVC is applied. In the proposed localized pre-contact workflow, ALS first identifies the hotspot, targeted dual-wavelength (UVC/UVA) radiation is applied before the surface is touched, and physical cleaning then removes the treated organic material.

By integrating optical detection, synergistic dual-wavelength decontamination, physical cleaning, verification, and documentation into a single quality-assurance process, organizations move from routine disinfection toward a more controlled and evidence-based hygiene strategy.

Chapter 6

Scientific Evidence Supporting Fluorescence-Based Hygiene Verification and UVC Decontamination

From Established Technologies to an Integrated Quality Assurance Framework

The scientific foundations of this white paper are derived from two well-established research fields: fluorescence-assisted optical inspection and ultraviolet-C (UVC) decontamination. Although these technologies have traditionally evolved independently, together they provide a complementary framework for objective hygiene verification.

The following sections summarize the current scientific evidence supporting environmental contamination assessment, fluorescence-assisted inspection, UVC decontamination, and integrated quality assurance.

6.1 Environmental Surface Contamination

Environmental surfaces contribute to the transmission of biological contamination in healthcare, hospitality, transportation, food service, and other public environments. Frequently touched objects accumulate organic residues through repeated human contact, creating predictable contamination hotspots.

While the presence of biological residues does not necessarily indicate pathogenic microorganisms, it provides evidence that cleaning is incomplete and that further attention is required.

6.2 Fluorescence-Based Inspection as a Quality Assurance Tool

Fluorescence imaging has been widely used in forensic science, biomedical research, and industrial inspection because it enables rapid visualization of naturally fluorescent materials without damaging the inspected surface.

In hygiene management, fluorescence should be regarded as a screening method rather than a diagnostic technique. It supports:

- rapid identification of residual biological material,
- objective documentation,

- inspection of large surface areas,
- immediate feedback for cleaning personnel,
- reproducible quality assurance.

Importantly, fluorescence imaging does not identify microorganisms or quantify infection risk. Its primary value lies in directing corrective cleaning and further investigation where appropriate.

6.3 Scientific Evidence Supporting UVC

The germicidal effectiveness of UVC irradiation has been demonstrated extensively across laboratory and clinical studies.

Published evidence consistently shows that microbial inactivation depends primarily on:

- delivered UVC dose,
- exposure geometry,
- surface characteristics,
- organic soiling,
- microorganism susceptibility.

These findings reinforce an important principle: UVC is most effective after adequate cleaning has removed materials that shield microorganisms from irradiation.

6.3.1 Recent Breakthroughs in Multi-Wavelength Synergy

Recent literature confirms that dual-wavelength applications outperform single-wavelength systems. A 2024 study published in *Water Research* evaluated the combined application of UV-A (365 nm) and UV-C (268 nm) using LED technology.

The researchers demonstrated that sublethal UV-A pre-exposure enhanced UV-C inactivation by several orders of magnitude. By tracing the molecular mechanism to UV-A-induced damage of transfer RNA (tRNA), the study proved that multi-wavelength systems effectively disable the pathogen's protein synthesis, preventing post-exposure cellular repair and eliminating the risk of pathogen regrowth.

6.3.2 Synergistic Destruction of Resilient Biological Proteins

In addition to preventing new protein synthesis, dual-wavelength irradiation actively accelerates the destruction of existing, highly stable biological proteins. Research conducted by Phoseon Technology evaluated the inactivation of RNase A, a notoriously denaturation-resistant enzyme.

The researchers designed the exposure to target specific structural vulnerabilities: the 275 nm UVC wavelength was used to disrupt disulfide bonds, while the 365 nm UVA wavelength targeted lysine amino acid side chains. The study demonstrated that 365 nm UVA light alone had no effect on the enzyme's activity, even at extreme doses up to 2400 J/cm².

However, when the target was exposed to the 275 nm and 365 nm sources concurrently, the effects were highly synergistic. The simultaneous application of both wavelengths dropped the required low-irradiance UVC dose for enzyme inactivation from 22.5 J/cm² down to just 5.6 J/cm². This confirms that utilizing multiple targeted wavelengths is a highly viable and rapid path for controlling and inactivating resilient biological proteins that would otherwise withstand single-wavelength treatments.

6.4 Lessons from Healthcare and the COVID-19 Pandemic

Healthcare has generated the strongest body of evidence for environmental UVC applications. Numerous studies have demonstrated reductions in microbial contamination when UVC is integrated into existing cleaning protocols.

During the COVID-19 pandemic, interest in UVC technology expanded rapidly. Independent investigations confirmed its effectiveness against many enveloped viruses under controlled exposure conditions while also highlighting several practical limitations:

- sufficient UVC dose is essential;
- surface cleanliness influences effectiveness;
- shadowed areas receive reduced irradiation;
- UVC complements, rather than replaces, routine cleaning.

These observations reinforced established principles rather than introducing new ones.

6.5 Human Factors and Cleaning Performance

Cleaning quality is influenced not only by procedures but also by human performance. Time pressure, staff turnover, experience, workload, and inconsistent supervision contribute to variability in cleaning outcomes.

Objective visual feedback reduces this variability by enabling personnel to identify missed areas immediately. Consequently, fluorescence-assisted inspection serves both as a verification method and as an effective training tool that supports continuous improvement.

6.6 An Integrated Quality Assurance Model

Quality management systems are based on a common sequence:

1. Inspection
2. Measurement
3. Corrective action
4. Verification

These principles are reflected in internationally recognised methodologies such as PDCA, ISO 9001, and continuous improvement frameworks.

Rather than introducing an entirely new concept, the ALS/UVC platform applies established quality management principles to environmental hygiene.

6.7 Remaining Research Opportunities

Although both fluorescence-assisted inspection and UVC decontamination are supported by substantial scientific evidence individually, several important research questions remain.

Future work should investigate:

- correlation between fluorescence intensity and ATP measurements;
- correlation between fluorescence findings and microbiological cultures;
- optimisation of ALS wavelengths for hygiene applications;
- automated image analysis using artificial intelligence;
- standardised contamination scoring systems;
- long-term operational and economic benefits.

Addressing these questions will contribute to the development of internationally standardised protocols for evidence-based hygiene verification.

6.8 Conclusions

Current scientific evidence supports several key conclusions.

- Environmental contamination remains an important component of hygiene management.
- Visual inspection alone cannot reliably verify biological cleanliness.
- Fluorescence-assisted inspection provides rapid, objective visualisation of residual biological material.
- UVC is a scientifically established method for non-chemical microbial inactivation when applied under validated conditions.
- Integrating these complementary technologies within a structured quality assurance process strengthens cleaning verification, documentation, staff training, and continuous improvement.

Taken together, the available evidence supports a transition from appearance-based inspection toward measurable, evidence-based hygiene verification.

The preceding chapters have established the scientific basis for fluorescence-assisted inspection and UVC decontamination. The following chapter translates these principles into practice by presenting the findings of a pilot evaluation conducted in real-world public restroom environments.

Chapter 7

Pilot Evaluation

Visualization of Residual Biological Contamination in Public Restrooms

From Laboratory Science to Real-World Application

The preceding chapters established the scientific basis for fluorescence-assisted inspection and UVC decontamination. To evaluate the practical applicability of these principles, a pilot field assessment was conducted using a forensic-grade Alternate Light Source (ALS) system in public restroom facilities.

The objective was not to identify microorganisms or assess infection risk. Instead, the pilot examined whether naturally occurring biological residues could be visualized following routine cleaning, whether contamination patterns could be identified, and whether ALS inspection could be integrated into routine hygiene verification without disrupting facility operations.

7.1 Objectives

The pilot was designed to address four operational questions:

1. Can ALS reveal biological residues invisible under normal lighting?
2. Which surfaces consistently exhibit residual fluorescence?
3. Can ALS inspection be integrated into routine facility operations?
4. Does fluorescence imaging provide actionable information for cleaning personnel?

Rather than replacing microbiological testing, the pilot evaluated the feasibility of fluorescence-assisted inspection as a rapid screening method for quality assurance.

7.2 Methodology

The assessment was conducted in public restroom facilities representing typical high-traffic environments. All inspected locations had undergone routine cleaning before examination.

Inspection was performed using:

- forensic-grade Alternate Light Source
- optical barrier filters
- digital photographic documentation

- standardized inspection procedure

The study was intentionally observational. No microbiological cultures, ATP measurements, or quantitative fluorescence measurements were performed. Consequently, the findings should be interpreted as qualitative observations rather than microbiological evidence.

7.3 Inspection Areas

Category	Typical Inspection Points
Entry	Door handles, push plates
Toilets	Flush buttons, seats, lids
Hand washing	Faucets, soap dispensers
Drying	Paper dispensers, hand dryers
Waste	Bin lids
Miscellaneous	Grab rails, wall switches

7.4 Observations



Figure 3. Evidence-based hygiene verification workflow. A surface that appears visually clean under conventional lighting often retains invisible biological residues. ALS inspection reveals fluorescent contamination hotspots. In the proposed localized workflow, the identified hotspot receives targeted UVC before physical cleaning; ALS reinspection then verifies whether fluorescent residue has been removed. Fluorescence indicates residual biological or

organic material, not the direct presence of pathogens. The pre-contact sequence is a product-specific operational approach requiring validation under Appendix L.

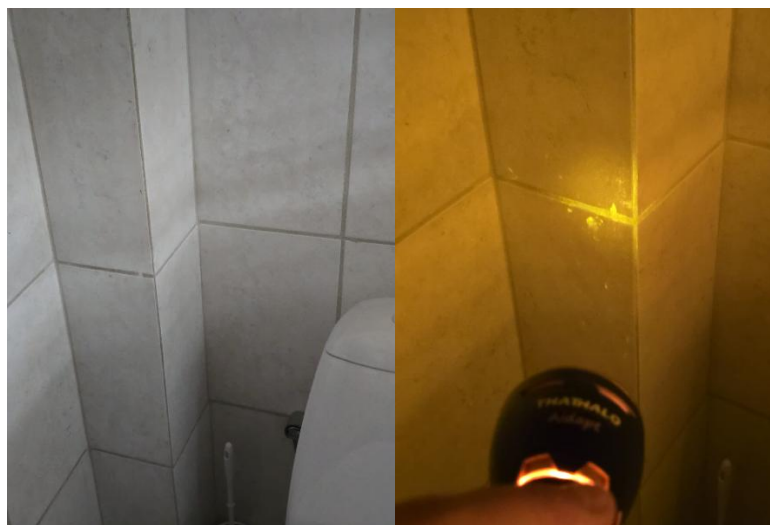
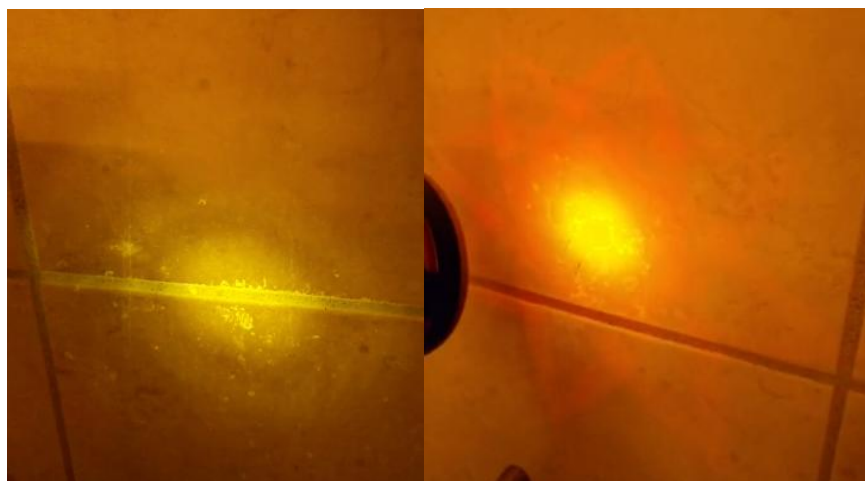


Figure 4. Comparison of conventional white-light inspection and Alternate Light Source (ALS) fluorescence imaging. Under conventional white-light illumination, the tiled wall appears visually clean. Under ALS illumination using an appropriate excitation wavelength and optical barrier filter, localized fluorescent residues become visible. These signals indicate residual biological or organic material and do not identify microorganisms or infection risk.



Visualization of Residual Contamination and Subsequent UVC Decontamination

Figure 5. Visualization of residual contamination and proposed pre-contact UVC intervention. The left image illustrates fluorescence-assisted visualization of residual biological or organic material using ALS. The localized signal identifies an area for targeted intervention.

The right image illustrates targeted UVC application to the detected hotspot before physical cleaning. This proposed pre-contact step is intended to reduce viable microorganisms before the surface is touched, thereby limiting the potential for transfer through hands, gloves, cloths, or cleaning equipment.

Representative ALS Findings from Public Fast-Food Restaurant Restrooms

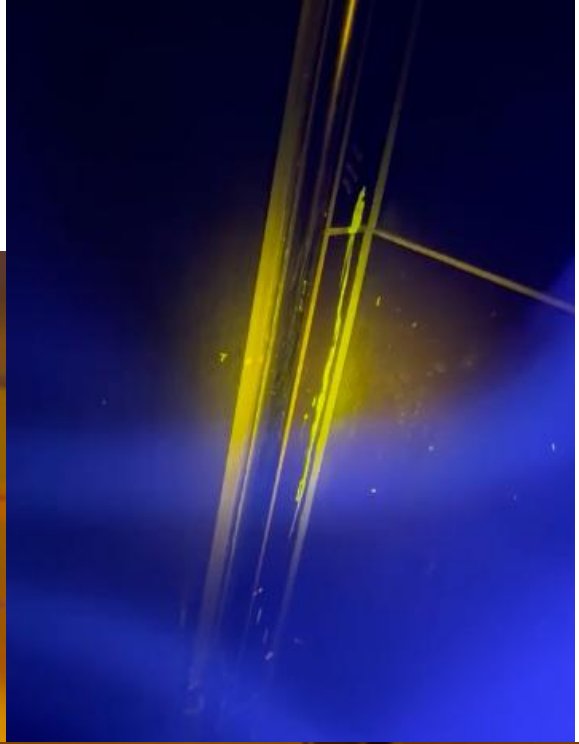
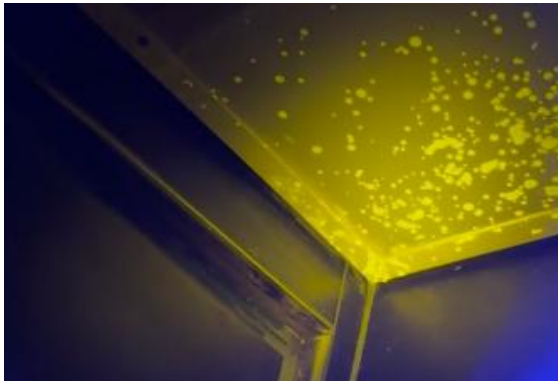
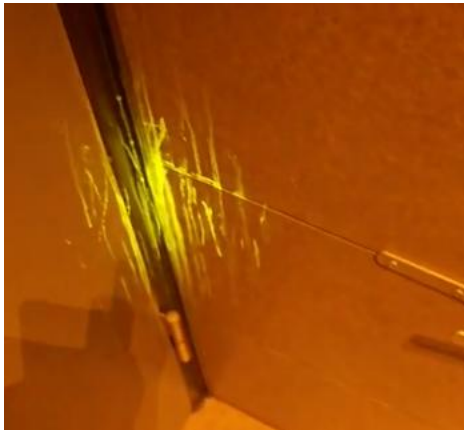
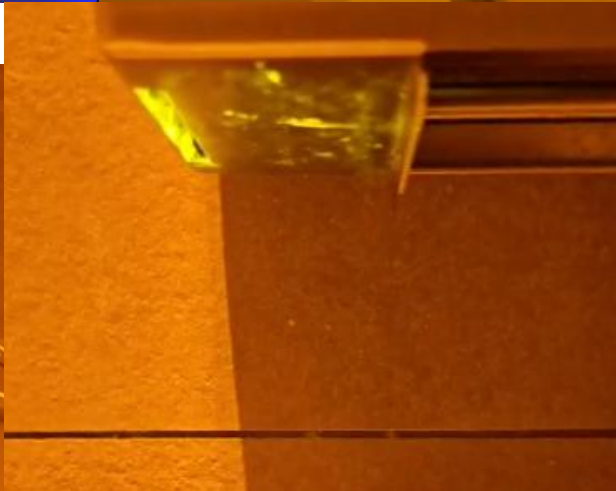
All photographs presented in this white paper were acquired during real-world field evaluations conducted in publicly accessible restroom facilities. Images have been anonymized and are presented exclusively for scientific and educational purposes to illustrate fluorescence-assisted hygiene verification. No conclusions regarding the overall hygiene standards of individual establishments should be inferred from these representative examples.

The images shown in this figure were captured during the pilot evaluation in restroom facilities of several public fast-food restaurants. They illustrate representative examples of fluorescence-assisted inspection using a forensic-grade Alternate Light Source (ALS).

Although the inspected surfaces generally appeared visually clean under conventional lighting, ALS examination revealed localized fluorescent residues on frequently touched and difficult-to-clean areas, including door frames, hinge assemblies, corners, and concealed structural elements. These locations are not routinely emphasized during visual inspections but accumulate residual biological or organic material over time.

The observed fluorescence patterns demonstrate that contamination is often concentrated in narrow crevices, joints, and recessed surfaces where routine cleaning is less effective. Such areas can therefore serve as practical indicators for evaluating cleaning performance and identifying opportunities for targeted corrective cleaning. Supporting images and qualitative heat maps are provided in Appendices D and E.

The images further illustrate the value of ALS technology in identifying contamination hotspots that remain invisible during conventional visual inspection, thereby providing objective guidance for evidence-based hygiene verification.



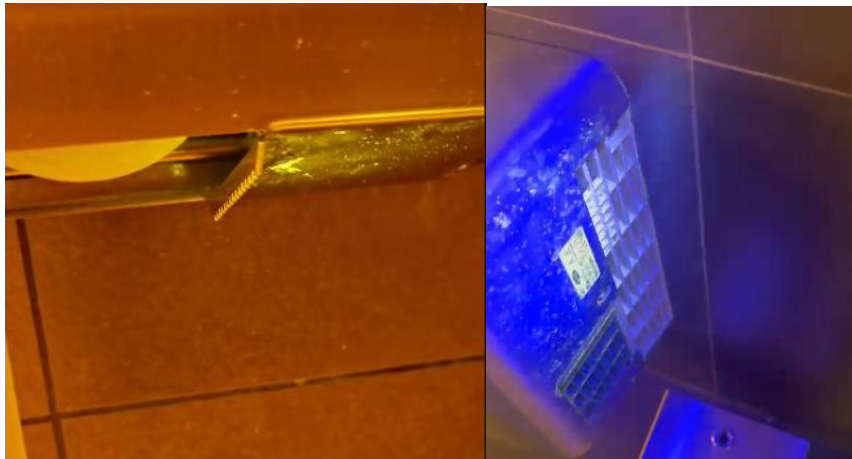


Figure 6. Representative ALS inspection findings from public fast-food restaurant restrooms. Fluorescence imaging revealed localized residual biological or organic material on high-touch and difficult-to-access surfaces that appeared visually clean under conventional lighting.

7.5 Contamination Distribution

Surface	Relative Fluorescence
Flush Button	Very High
Door Handle	High
Faucet	High
Soap Dispenser	Medium
Toilet Paper Holder (underside)	Very High
Wall Tiles	Low
Floor	Low

7.6 Operational Observations

- Inspection required only a few minutes.
 - No interruption of facility operation.
 - Documentation was immediate.
 - Fluorescence hotspots were reproducible.
 - Cleaning personnel immediately understood the findings.
-

7.7 Limitations

The present pilot has several important limitations:

- qualitative observations only;
- no ATP comparison;
- no microbiological confirmation;
- limited number of inspected facilities;
- no statistical analysis;
- no longitudinal evaluation.

Accordingly, the findings should be interpreted as proof of operational feasibility rather than quantitative validation.

7.8 Lessons Learned

The pilot demonstrated several important operational principles:

- visual appearance alone is a poor predictor of residual biological contamination;
- high-touch surfaces consistently represent contamination hotspots;
- objective visual feedback supports quality assurance and staff training.

Most importantly, ALS shifts hygiene verification from a subjective judgement—

"Does this surface look clean?"

—to an evidence-based question—

"Where does residual biological material remain?"

7.10 Conclusions

The pilot demonstrated that forensic-grade Alternate Light Source technology can reliably visualize residual biological contamination that remains invisible under conventional lighting.

Although the study was not designed to identify microorganisms or quantify infection risk, it demonstrated that fluorescence-assisted inspection can be integrated into routine hygiene verification with minimal operational impact.

These findings support the concept that ALS provides a valuable additional layer of quality assurance by enabling rapid identification of contamination hotspots, objective documentation, and immediate feedback for cleaning personnel.



Figure 7. Conceptual heat map of residual biological contamination detected by Alternate Light Source (ALS) inspection. Red areas indicate locations where residual fluorescence was most consistently observed during the pilot evaluation, primarily on high-touch surfaces. The colour scale represents relative fluorescence intensity only and should not be interpreted as microbial load or infection risk. This conceptual figure summarizes qualitative observations rather than quantitative measurements.

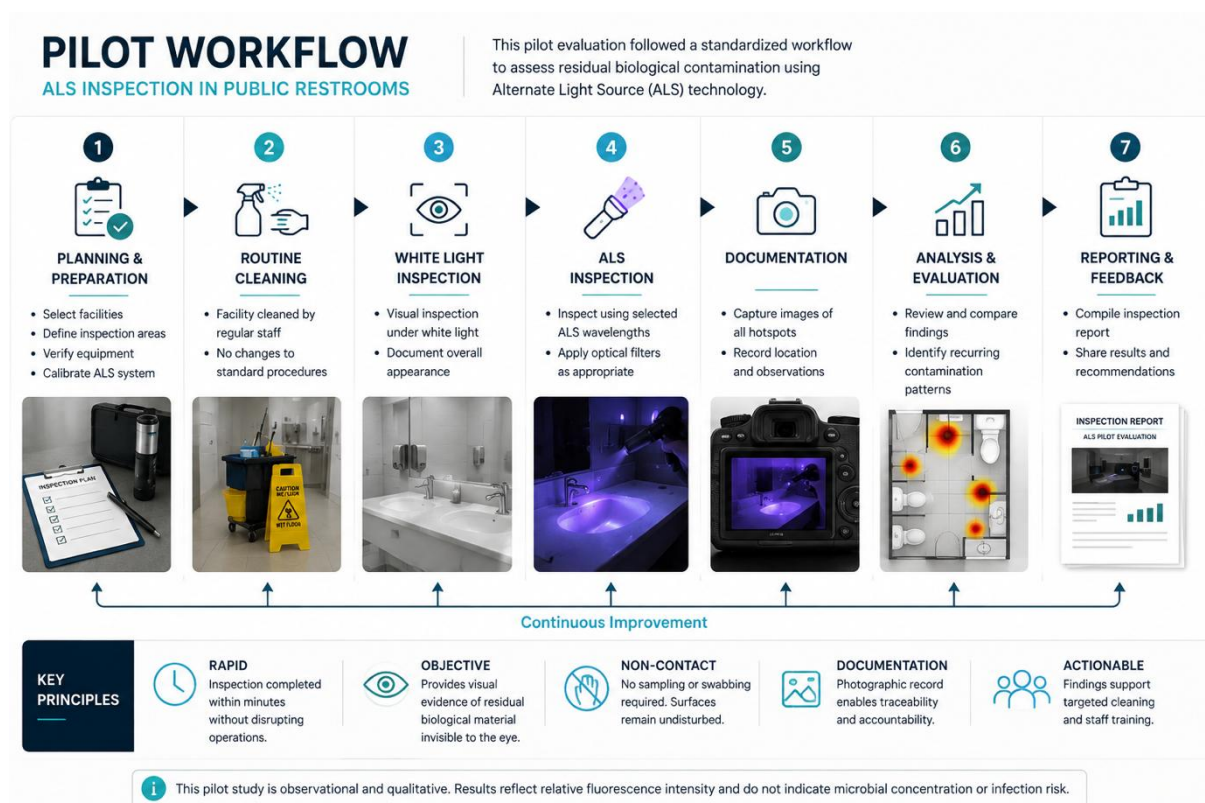


Figure 8. Pilot evaluation workflow illustrating inspection, hotspot identification, corrective action, verification, and continuous improvement.

Workflow Description

The pilot evaluation followed a standardized seven-stage workflow designed to assess residual biological contamination in public restroom facilities using Alternate Light Source technology (see Appendix A).

The process began with **planning and preparation**, including the selection of facilities, definition of inspection areas, verification of equipment, and preparation of the ALS system. The facilities then underwent **routine cleaning by regular personnel**, without changes to the established cleaning procedures. This ensured that the evaluation reflected normal operational conditions.

Following cleaning, each inspection area was first examined under **conventional white light** to document its general appearance and identify any visible contamination. The same surfaces were then assessed using selected **ALS wavelengths and appropriate optical barrier filters** to reveal fluorescent residues that were not visible under normal illumination.

All relevant findings were photographed and recorded during the **documentation stage**, including the location, surface type, and observed fluorescence pattern. The documented findings were subsequently reviewed to identify recurring contamination hotspots and compare the results of white-light and ALS inspection.

The final stage involved compiling the observations into an inspection report and communicating the findings to relevant personnel. The results then supports targeted corrective cleaning, staff training, and refinement of future inspection procedures.

This workflow was designed as a continuous improvement cycle. Findings from each inspection can inform subsequent cleaning priorities, inspection planning, and quality assurance activities.

Chapter 8

Business Case

Transforming Hygiene Verification into a Strategic Advantage

From Operational Cost to Strategic Value

Historically, hygiene has been regarded as an operational necessity—a cost associated with cleaning staff, consumables, facility maintenance, and regulatory compliance. Success was typically measured by whether cleaning tasks were completed on schedule and whether facilities appeared clean.

Today, this perspective is changing. Organizations increasingly recognize that hygiene directly influences customer confidence, employee wellbeing, operational resilience, regulatory compliance, and brand reputation. Consequently, cleaning is evolving from a routine operational function into a measurable component of organizational quality management.

The integration of **Alternate Light Source (ALS) inspection** and **Ultraviolet-C (UVC) decontamination** supports this transition by providing objective evidence of cleaning effectiveness rather than relying solely on visual appearance.

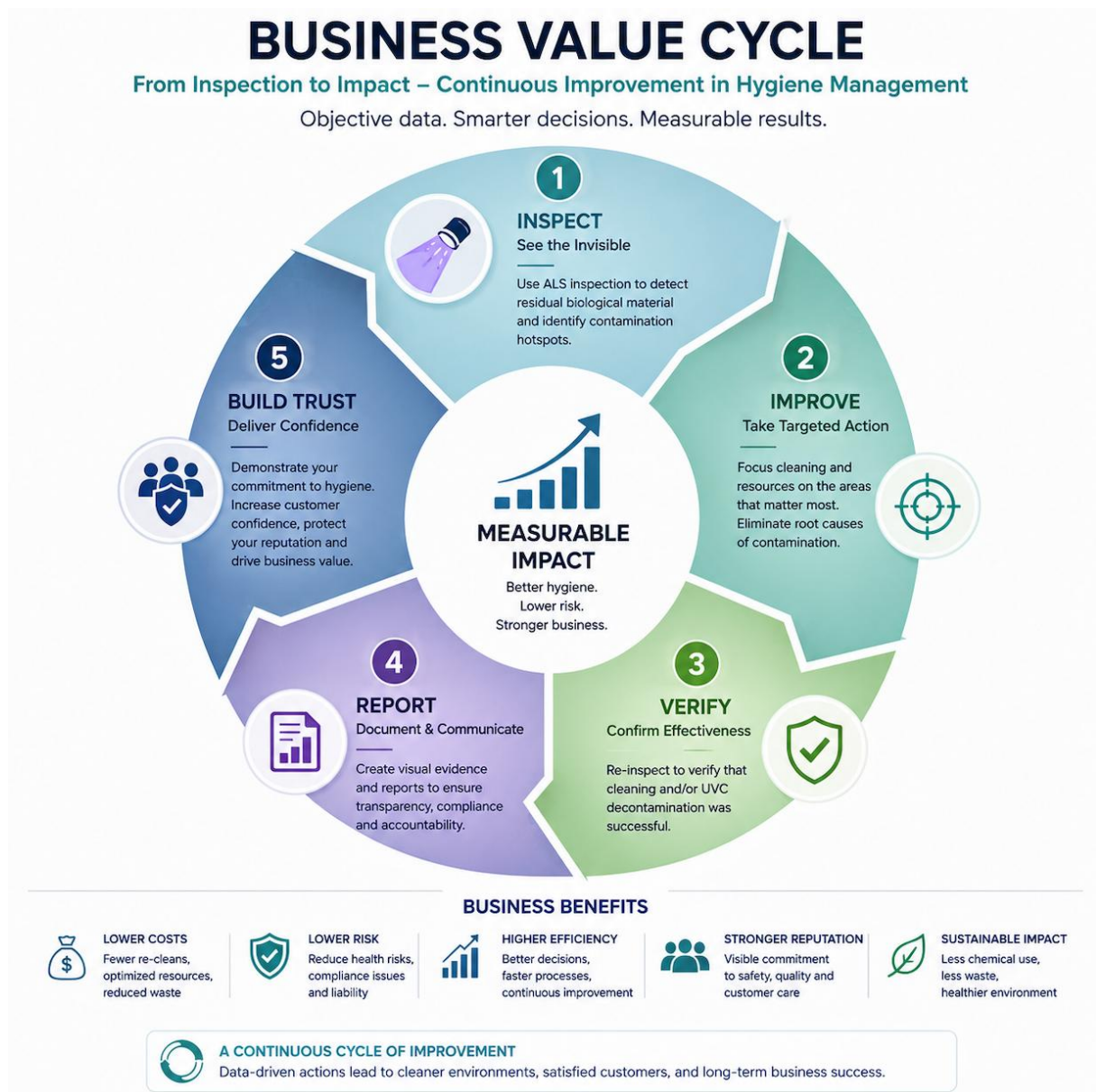


Figure 11. Business value cycle linking detection, intervention, verification, documentation, and continuous improvement.

8.1 The Cost of Poor Hygiene

The consequences of inadequate hygiene extend far beyond visible cleanliness.

Potential impacts include:

- customer complaints;
- reputational damage;
- regulatory findings;
- increased absenteeism;

- healthcare-associated infections;
- food safety incidents;
- contract disputes;
- repeat cleaning;
- loss of customer confidence.

Although the financial impact varies by sector, indirect costs frequently exceed the direct costs of cleaning itself.

A single hygiene-related incident affects customer retention, operational continuity, and public trust far more than the cost of preventive quality assurance.



Figure 10. Cost-of-poor-hygiene pyramid showing direct, indirect, and strategic impacts.

8.3 Objective Quality Assurance

Most organizations currently document hygiene through:

- cleaning schedules;
- supervisor inspections;
- contractor reports;
- ATP measurements;
- microbiological sampling.

These methods demonstrate that activities have been performed but often provide limited visual evidence of cleaning outcomes.

ALS inspection adds a new level of transparency by enabling photographic documentation of inspected surfaces.

Instead of documenting:

Cleaning completed.

Organizations can document:

Surface inspected following cleaning. No residual fluorescence observed.

This distinction strengthens auditability, accountability, and confidence in cleaning performance.

8.4 Optimising Cleaning Efficiency

Cleaning resources—including personnel, time, chemicals, and budgets—are inherently limited.

Objective inspection allows organizations to:

- focus effort on contamination hotspots;
- reduce unnecessary repeat cleaning;
- improve allocation of labour;
- optimise inspection frequency;
- prioritise high-risk surfaces.

Rather than increasing cleaning effort, ALS inspection supports **smarter resource allocation** through evidence-based decision making.

8.5 Supporting Personnel and Contractors

Objective visual feedback provides immediate educational value.

For cleaning personnel, fluorescence-assisted inspection:

- improves understanding of contamination patterns;
- accelerates practical training;
- reinforces correct cleaning techniques;
- reduces performance variability.

For facility managers and contractors, documented inspection results create transparent performance metrics, reduce disputes, and support evidence-based service evaluation.

8.6 Regulatory and Compliance Benefits

Across many industries, organizations are expected to demonstrate that hygiene procedures are implemented effectively.

ALS documentation can strengthen:

- internal quality audits;
- regulatory inspections;
- customer audits;
- contractor verification;
- ISO-based quality management systems;
- HACCP documentation where applicable.

The technology does not replace existing compliance procedures but provides additional objective evidence supporting them.

8.7 Sustainability

Evidence-based inspection also contributes to environmental sustainability.

Potential benefits include:

- reduced chemical consumption;
- fewer unnecessary repeat cleaning cycles;
- lower use of disposable testing materials;
- improved water efficiency;
- more targeted application of disinfectants.

Similarly, UVC enables microbial reduction without leaving chemical residues. When integrated appropriately, this reduces dependence on chemical disinfectants while maintaining hygiene performance.

8.8 Return on Investment (ROI)

The economic value of ALS/UVC implementation extends beyond labour savings. An illustrative calculation framework is provided in Appendix H.

Potential benefits include:

Benefit	Operational Impact
Reduced repeat cleaning	Lower labour costs
Targeted inspections	Improved resource allocation
Faster staff training	Reduced onboarding time

Benefit	Operational Impact
Objective documentation	Fewer contractor disputes
Improved compliance	Lower audit risk
Enhanced customer confidence	Stronger brand reputation
Early detection	Reduced operational risk

The financial return will depend on facility type, inspection frequency, and implementation strategy. However, the overall value proposition extends beyond equipment acquisition costs by improving efficiency, transparency, and risk management.

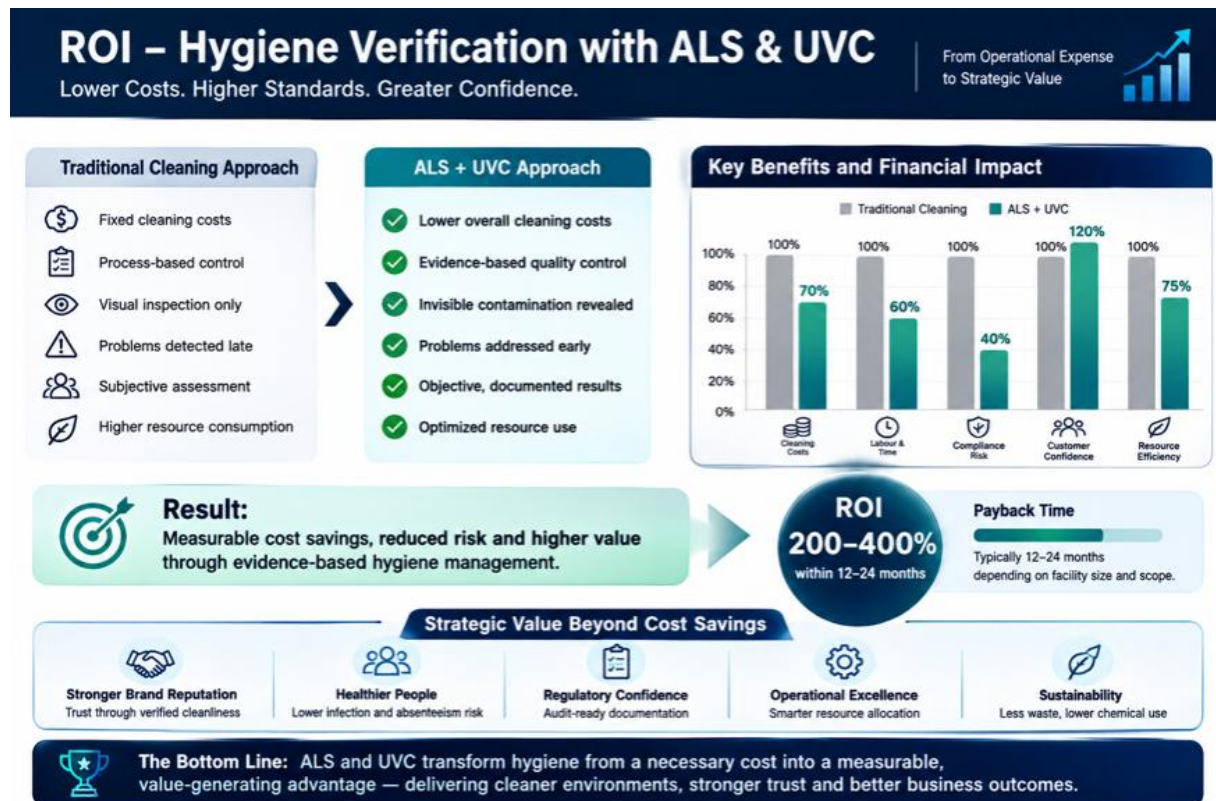


Figure 9. Illustrative return-on-investment comparison for hygiene verification with ALS and UVC.

8.9 Towards a New Standard

Historically, organizations measured hygiene by process:

- Was the room cleaned?
- Was the checklist completed?
- Was the inspection signed?

Evidence-based hygiene management measures outcomes:

- Was residual contamination detected?
- Was decontamination completed?
- Was corrective cleaning performed?
- Was the result objectively verified?

This shift mirrors developments in other quality-driven industries, where subjective assessment has progressively been replaced by measurable performance indicators.

8.10 Vision: From Clean to Verified Clean

The future of hygiene management is likely to move beyond perceived cleanliness toward **verified cleanliness**.

The integrated ALS/UVC platform enables organizations to:

- Detect invisible contamination.
- Document objective findings.
- Support corrective cleaning.
- Verify cleaning effectiveness.
- Continuously improve operational performance.

Rather than viewing hygiene as a routine maintenance task, organizations can treat it as a measurable component of quality, safety, and customer experience.

Chapter 9

Implementation Framework

From Pilot Project to Operational Standard

Evidence-based hygiene verification creates operational value especially when it is integrated into existing cleaning procedures, responsibilities, documentation systems and quality-management processes. The framework complements established hygiene programmes and should be adapted to each organisation's facility type, risk profile, operational capacity and regulatory requirements.

9.1 Phased Implementation

Implementation should follow four stages:

1. **Baseline assessment:** Identify high-touch and critical surfaces, review current cleaning and inspection procedures, define objectives and select suitable pilot areas.
2. **Pilot implementation:** Introduce ALS inspection in a controlled environment to identify recurring fluorescence hotspots, assess operational feasibility and refine documentation. Where UVC is used, exposure conditions and safety procedures must be defined and validated.
3. **Standardisation:** Establish procedures for inspection, image documentation, classification of findings, targeted UVC application, corrective cleaning, verification and escalation.
4. **Operational integration and scaling:** Assign ownership, establish risk-based inspection schedules, monitor KPIs and expand the programme only after operational value has been demonstrated.

9.2 Operational Workflow

The routine process should remain simple and repeatable:

The inspection area, frequency and responsible personnel are defined before an initial white-light and Purple-light assessment. Relevant findings are documented, followed by validated targeted UVC treatment before physical contact. Corrective cleaning removes remaining organic material, and Purple-light reinspection verifies the visible result. Recurring findings are analysed to improve cleaning priorities, procedures and training.

9.3 Responsibilities

Management provides programme ownership and resources; hygiene or quality managers oversee procedures, KPIs and reporting; operators perform inspection, UVC treatment and documentation; cleaning personnel complete corrective actions; and health-and-safety personnel ensure compliance.

9.4 Documentation and Performance Measurement

Each inspection record should identify the facility, area, date, inspector, surface, images, finding classification, corrective action and verification status. Findings may be classified as no relevant fluorescence, low, moderate or high. This scale represents relative optical observations only.

Digital reporting enables traceability, hotspot mapping, trend analysis, audit preparation and comparison across facilities. Key indicators include inspection completion, hotspot recurrence, corrective-action closure time, verification success, improvement after training, contractor compliance, cleaning time and use of chemicals or consumables. Trends are more meaningful than isolated results; an initial increase in hotspots may reflect improved detection rather than poorer cleaning.

Chapter 10

Practical Implementation

From Scientific Concept to Operational Solution

Transitioning from scientific theory to real-world application requires a specialized hardware solution capable of seamlessly uniting optical detection, active decontamination, and digital verification into a single, intuitive workflow.

10.1 Closing the Gap Between Detection and Action

The practical value of fluorescence-assisted hygiene verification depends not only on scientific principles but also on the usability of the technology in everyday operational environments. For routine implementation, the inspection system must be portable, intuitive, and immediately deployable without requiring complex equipment or extensive technical training.

To address these requirements, a developer-described patent-protected integrated inspection platform has been developed that combines fluorescence-assisted detection and targeted UVC decontamination within a single handheld device. The system was specifically designed to enable rapid hygiene verification and immediate intervention during normal facility operation (Developer technical specification, 2026).

10.2 Integrated High-Intensity Handheld Platform

Thathalo AIdapt combines fluorescence-assisted detection, high-intensity UVC decontamination, post-cleaning verification and digital documentation in one compact, battery-powered handheld device.

Its patent-protected dual-technology architecture enables the complete hygiene-control workflow to be performed with a single portable platform

Rather than functioning as a conventional handheld UVC lamp, the system integrates contamination detection, targeted treatment, distance control, operator protection, verification and documentation into one operational platform.

Key Characteristics

- Integrated **Purple-light** detection and **Dual-Wavelength** (UVC/UVA) decontamination.
- **Simultaneous** delivery of 265 nm (35 mW/cm²) and 365 nm (1.5 W) at the treatment surface
- Patent-protected **dual-technology** architecture

- Precision-engineered dual **5° collimated optics** delivering simultaneous 265 nm UVC (35 mW/cm²) and 365 nm UVA (1.5 W) at a 10 cm working distance.
- Rapid and **concentrated UVC** dose delivery
- Integrated **Orange Halo Distance Guide**
- Gyroscope-controlled UVC **safety** interruption
- Compact handheld design weighing approximately **290 g**
- **Rechargeable** battery operation
- Smartphone-compatible **optical barrier filter**
- Orange inspection **safety glasses**
- App-supported workflow and digital **documentation**

High-Intensity Precision Dual-Wavelength Optics The specified irradiance is measured exactly at the treatment surface rather than at the LED emitter. This distinction is critical because distance, beam divergence, optical losses, and treatment geometry directly influence the total radiant energy reaching the target.

To guarantee that the dual-wavelength synergy operates correctly, the system utilizes a precision-engineered 5° narrow optical array. This design limits beam divergence and forces the 265 nm UVC and 365 nm UVA beams to perfectly overlap on the exact same localized footprint. By eliminating photon scattering, the optics concentrate both the direct genomic destruction (UVC) and the oxidative ROS blast (UVA) into a highly defined treatment area, ensuring rapid and irreversible pathogen inactivation.

No other identified portable handheld system combines this level of simultaneous dual-wavelength surface irradiance with such a narrowly focused, co-aligned optical delivery system.

Rapid UVC Dose Delivery

UVC dose is determined by irradiance and exposure time:

$$\text{UVC dose} = \text{irradiance} \times \text{exposure time}$$

Under stable, direct and unobstructed exposure conditions, the platform provides the following calculated incident doses:

Exposure time Incident UVC dose

1 second	35 mJ/cm ²
2 seconds	70 mJ/cm ²
3 seconds	105 mJ/cm ²
5 seconds	175 mJ/cm ²
10 seconds	350 mJ/cm ²

The high surface irradiance enables many defined target doses to be delivered within seconds rather than minutes, providing an important operational advantage where multiple contamination hotspots must be treated efficiently.

Orange Halo Distance Guide

Consistent UVC treatment requires reproducible positioning. The integrated **Orange Halo Distance Guide** provides immediate visual confirmation of the correct working distance.

As the device moves towards or away from the target surface, the projected orange ring changes in definition. When the halo appears sharply focused, the correct treatment position has been reached.

This converts a critical treatment parameter into a simple visual procedure, helping operators reproduce the defined treatment conditions without rulers, physical spacers or external measuring instruments.

Gyroscope-Controlled UVC Safety

An integrated gyroscope continuously monitors device orientation during UVC operation. If the device is lifted beyond its permitted operating orientation or directed towards the operator, UVC emission is automatically interrupted.

The focused optical system, visual distance control and automatic orientation monitoring therefore operate together as a coordinated safety and application system, supporting controlled and reproducible point-of-use treatment.

Competitive and Operational Significance

The platform integrates technologies that are normally provided by separate devices into one portable workflow. The operator can:

Detect contamination → Apply targeted UVC

This enables contamination hotspots to be identified and treated directly at the point of need without external cameras, laboratory equipment or separate UVC treatment devices.

No other identified handheld platform combines **Purple-light detection, high-intensity focused UVC treatment, visual distance control, automatic orientation safety, post-cleaning verification and digital documentation within a single system.**

The platform therefore represents more than an incremental improvement to conventional handheld UVC devices. It establishes an integrated approach in which biological contamination can be detected, treated, cleaned, verified and documented using one portable platform.

Parameter	Standard UVC (e.g., 254 nm / 265 nm)	Synergistic Dual-Wavelength (265 nm UVC + 365 nm UVA)
Primary Inactivation Mechanism	Relies entirely on direct photolysis; photons are absorbed by the spore's DNA/RNA, forming cyclobutane pyrimidine dimers to halt replication.	Operates via a simultaneous multi-pathway attack: UVC destroys the DNA, while UVA directly damages transfer RNA (tRNA) and generates Reactive Oxygen Species (ROS).
Effect on Proteins & Enzymes	Does not directly target or rapidly degrade the spore's existing protective proteins or repair enzymes.	Synergistically destroys resilient biological proteins and rapidly shuts down the synthesis of new proteins (via tRNA crosslinking).
Recovery & Photoreactivation	Surviving spores may eventually recover, repair their DNA, or germinate if the UVC dose is sublethal, relying on cellular repair enzymes.	Because the UVA wavelength fundamentally degrades the proteins and enzymes required for photoreactivation, the UVC-induced genomic damage becomes permanently irreversible.
Operational Application	Typically deployed as a supplementary, room-scale terminal intervention <i>after</i> physical cleaning is completed.	Designed as a localized, pre-contact intervention where targeted radiation treats the hotspot <i>before</i> it is physically touched or wiped.
Geometric Shadowing	Highly susceptible to shadowing; recessed, angled, or obstructed surfaces often receive an insufficient dose from stationary units.	Delivered via a handheld device with 5-degree collimated optics, allowing the operator to aim the concentrated beam directly into corners, edges, and crevices.
Expected Dose Requirements	Typical established doses range from 80 to 150 mJ/cm² to achieve a >99% reduction in highly resistant <i>C. difficile</i> spores.	While the device delivers 105 mJ/cm ² in three seconds, the dual-wavelength synergy is expected to significantly <i>lower</i> the total joules required to achieve spore inactivation, similar to how it drastically reduced the dose needed for RNase A.

Chapter 11

The Future of Evidence-Based Hygiene

Conclusions, Vision and Next Steps

Beyond Cleaning—Towards Verified Hygiene

Environmental cleanliness has traditionally been assessed through visual appearance. While this remains important, it provides limited information about residual biological contamination.

Cleaning should not only be performed—it should be assessed, corrected where necessary, verified and documented.

This represents a transition from perceived cleanliness to **evidence-based hygiene management**.

11.1 From Visual Inspection to Evidence-Based Verification

Hygiene verification has evolved from visual inspection and checklists toward scientific sampling methods such as microbiological culture and ATP testing. These methods provide valuable information but are generally limited to selected sampling locations.

ALS provides rapid, non-contact inspection across larger surface areas and can identify residual biological material and contamination hotspots.

The central question therefore changes from:

Was cleaning performed?

to:

Can cleaning effectiveness be objectively demonstrated?

11.2 Value Across Industries

The framework can be applied wherever high-touch surfaces and shared environments require consistent hygiene management, including:

- healthcare;
- hospitality;
- restaurants and food service;
- airports and public transportation;
- schools and public institutions;
- commercial buildings and shopping centres; and
- facility-management and cleaning services.

Implementation should always reflect sector-specific risks, materials, user populations and regulatory requirements.

11.3 Digital Hygiene Management

Digital documentation transforms individual inspections into structured organizational data.

A digital system can combine inspection images, surface identification, findings, corrective actions, verification status and recurrence history. Aggregated data can support:

- contamination heat maps;
- trend analysis;
- facility comparisons;
- contractor-performance monitoring;
- management dashboards;
- risk-based inspection schedules; and
- audit preparation.

Instead of isolated inspection records, organizations gain a traceable dataset showing **where contamination occurs, how frequently it returns and whether corrective measures are effective**.

11.4 Artificial Intelligence

Artificial intelligence can further strengthen fluorescence-assisted inspection through automated image analysis, intensity classification, hotspot recognition, longitudinal comparison and predictive cleaning recommendations.

AI should support rather than replace human interpretation.

11.5 Scientific Opportunities

Future research should therefore examine:

- correlation with ATP measurements and microbiological cultures;
- sensitivity and specificity for different residue types;
- inter-operator repeatability;
- effects of surfaces and cleaning products;
- quantitative fluorescence scoring;
- microbiological effectiveness of the integrated workflow;
- long-term cleaning-performance improvement; and
- operational cost-effectiveness.

Multicentre studies across different industries would provide a stronger basis for standardized protocols and performance benchmarks.

11.6 Towards International Standards

Broader adoption requires standardized methods for equipment performance, wavelength and irradiance verification, optical filters, inspection geometry, image acquisition, fluorescence classification, UVC dose verification, safety controls, operator competency and digital reporting.

Future standards should clearly distinguish between:

- detection of residual organic material;
- measurement of microbial contamination;
- assessment of infection risk; and
- verification of cleaning effectiveness.
-

11.7 From Inspection to Continuous Improvement

The greatest value of evidence-based hygiene verification lies not in identifying individual cleaning failures, but in recognizing recurring patterns and improving processes.

Inspection data can reveal weaknesses related to cleaning methods, facility design, staff training, workload and surface selection. Used constructively, this information supports collaboration between cleaning personnel, facility managers, contractors and quality teams.

The objective is a cultural shift from **assumption to evidence, routine completion to verified outcomes, and reactive correction to continuous improvement.**

11.8 Final Conclusions

The analysis presented in this white paper supports five principal conclusions:

1. **Visual cleanliness alone cannot verify the absence of residual biological material.**
2. **ALS enables rapid, non-contact visualization of fluorescent residues but does not identify microorganisms, quantify microbial load or determine infection risk.**
3. **Targeted UVC provides microbial inactivation under defined conditions, while physical cleaning remains necessary to remove organic material.**
4. **Integrating detection, decontamination, cleaning, verification and documentation creates a structured and traceable hygiene-control process.**
5. **Further quantitative and multicentre validation is required to establish standardized protocols, microbiological performance and broader implementation criteria.**

11.9 Closing Perspective

The future of hygiene management will not be determined simply by how frequently surfaces are cleaned, but by how effectively contamination is identified, corrective action is targeted and outcomes are verified.

Thathalo AIdapt makes residual biological material visible. Targeted UVC reduces viable microorganisms on directly exposed hotspots before physical contact. Cleaning removes the treated material, verification assesses the result, and digital documentation makes the process traceable.

The objective is not to claim absolute cleanliness, but to establish a more transparent, measurable and continuously improving hygiene process.

The future of hygiene is not simply clean. It is verified, documented and continuously improved.

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Suggested Figures (Adapted from Literature)

- Jablonski Diagram (adapted from Lakowicz, 2023)
 - Fluorescence excitation/emission spectra of endogenous fluorophores (adapted from Croce & Bottiroli, 2014)
 - Electromagnetic spectrum (adapted from ISO/ASTM educational resources)
 - UV dose-response curves (adapted from Kowalski, 2009)
-

Image Credits

Unless otherwise stated, all pilot photographs, ALS inspection images, fluorescence documentation, contamination heat maps, workflow diagrams, and business graphics were created by the author.

Scientific figures adapted from published literature are reproduced or redrawn with appropriate attribution and in accordance with the applicable publisher licence.

APPENDICES

The appendices provide supplementary technical information supporting the methodology, scientific background, pilot evaluation, and practical implementation described throughout this white paper. They are intended to enhance transparency, reproducibility, and operational applicability without interrupting the flow of the main document. Cross-references are included throughout the main text so that scientific claims, field observations, safety requirements, dose guidance, and validation proposals can be traced directly to the relevant supporting appendix.

Appendix A

Pilot Evaluation Methodology

Objective

The pilot evaluation was designed to assess the operational feasibility of fluorescence-assisted hygiene verification using a forensic-grade Alternate Light Source (ALS) system in public restroom environments. The full study method is provided in Appendix A; structured observations and supporting images are provided in Appendices C–E.

The objectives were to determine whether:

- residual biological material was visualized after routine cleaning,
- recurring contamination hotspots were identified,
- ALS inspection was integrated into normal cleaning workflows,
- objective documentation supports quality assurance and staff training.

Equipment

- Thathalo AIdapt, handheld purple/UVC device
- Multiple excitation wavelengths
- Optical barrier filters
- Digital camera
- Standard inspection forms

Inspection Procedure

1. Routine cleaning completed
2. White-light inspection
3. ALS inspection
4. Image documentation
5. Evaluation of findings
6. Corrective cleaning (where required)
7. Verification

Study Limitations

- Qualitative observations only
- No ATP measurements
- No microbiological cultures
- No statistical analysis
- Limited number of inspection sites

Appendix B

ALS Inspection Checklist

Inspection Item	Completed	Comments
White-light inspection	☐	
ALS inspection	☐	
Correct barrier filter selected	☐	
Photographs captured	☐	
Fluorescence observed	☐	
Corrective cleaning performed	☐	
UVC treatment applied (if required)	☐	
Verification completed	☐	
Report finalized	☐	

Appendix C

Pilot Observation Tables

Surface	White-Light Appearance	ALS Finding	Recommended Action
Flush button	Clean	High fluorescence	Corrective cleaning
Faucet handles	Clean	Moderate fluorescence	Corrective cleaning
Door handle	Clean	High fluorescence	Corrective cleaning
Soap dispenser	Clean	Low fluorescence	Monitor
Toilet paper holder (underside)	Clean	Very high fluorescence	Immediate cleaning
Waste-bin lid	Clean	Moderate fluorescence	Corrective cleaning

Appendix D

Visual Documentation of the Integrated Hygiene-Control Workflow

This appendix provides supplementary photographic documentation illustrating the practical application of the integrated hygiene-control workflow described in Chapter 10.

The documentation follows the standardized operational sequence:

Detect → Decontaminate → Clean → Verify → Document

1. Detect – Purple-Light Inspection

The surface is inspected using Purple light to identify fluorescent biological or organic residues that are not readily visible under conventional white-light inspection.

2. Decontaminate – Targeted UVC Treatment

The identified hotspot is subjected to targeted UVC treatment before physical cleaning. The Orange Halo Distance Guide is used to establish the defined 10 cm working distance. Treatment time is determined by the validated target dose and specified surface irradiance.

3. Clean – Physical Removal

Following UVC treatment, the surface is physically cleaned using the applicable facility cleaning procedure. This step removes organic material and other residues that UVC treatment does not physically remove.

4. Verify – Purple-Light Reinspection

The treated and cleaned surface is reinspected using Purple light. The before-and-after images provide visual documentation of whether fluorescent residue remains and whether additional corrective cleaning is required.

5. Document – Digital Record

The completed intervention is documented photographically and, where applicable, recorded through the digital application. The record may include the surface location, initial finding, UVC treatment, cleaning action, verification result, date, time and operator.

Recommended Figure Structure

Each documented example should preferably be presented as a five-stage sequence:

Figure D-1. Example integrated hygiene-control workflow – high-touch surface

A – DETECT: Purple-light image showing the initial fluorescent hotspot.

- B – DECONTAMINATE: Targeted UVC application at the defined working distance.
- C – CLEAN: Physical cleaning of the treated surface.
- D – VERIFY: Purple-light reinspection following cleaning.
- E – DOCUMENT: Final digital record of the completed intervention.

Where available, a conventional white-light image may additionally be shown alongside the initial Purple-light image to demonstrate the difference between visually clean and fluorescence-assisted inspection.

Interpretation of Images

Fluorescence observed during Purple-light inspection indicates the presence of fluorescent biological or organic material. It does not, by itself, demonstrate the presence of viable microorganisms or specific pathogens.

Similarly, photographs of UVC treatment illustrate the application procedure but do not constitute microbiological evidence of microbial inactivation. Microbiological effectiveness must be established through controlled validation under the procedures described in Appendix L.

The photographic sequence therefore documents the operational workflow and visible cleaning outcome, while microbiological performance remains subject to independent quantitative validation.

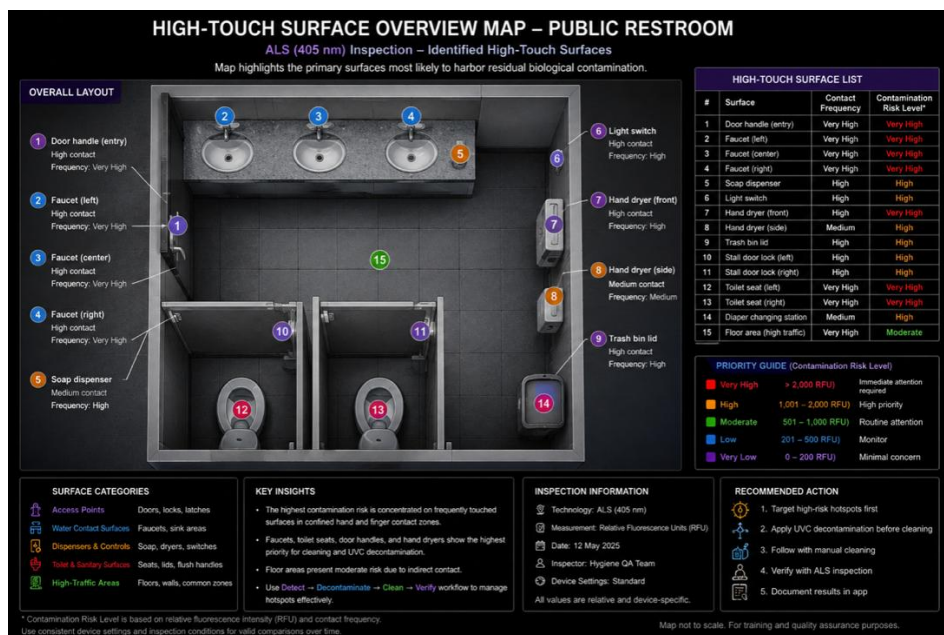
Appendix E

Contamination Heat Maps

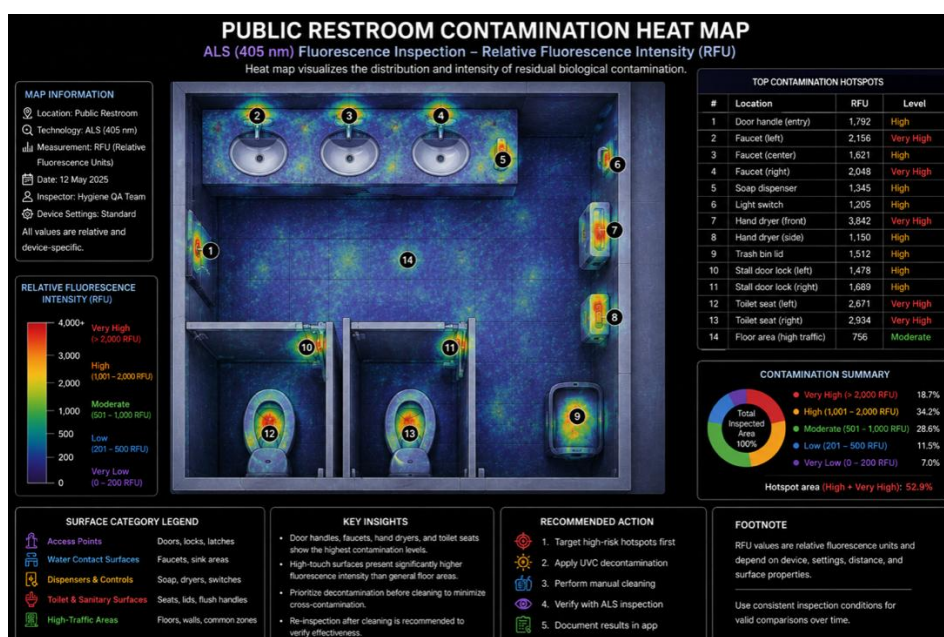
This appendix summarizes the spatial distribution of fluorescence observed during the pilot evaluation.

Included figures:

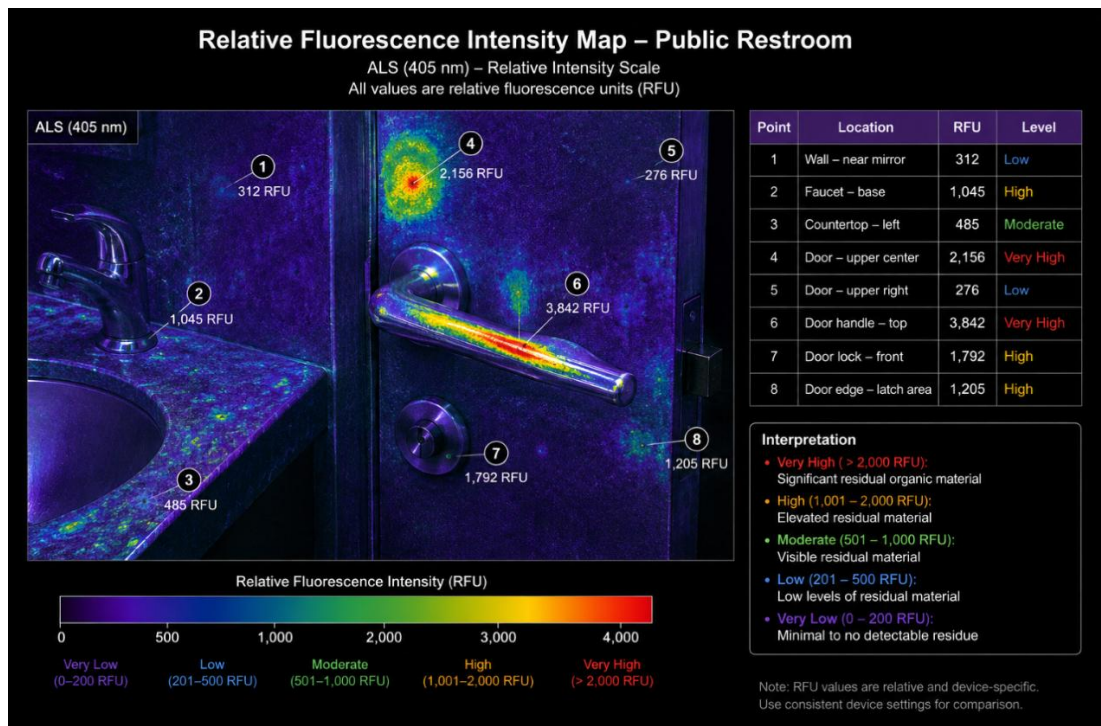
- Public restroom contamination heat map



- High-touch surface overview



- Relative fluorescence intensity map



- Contamination hotspot distribution



Important Note

The heat maps represent qualitative fluorescence observations and do not indicate microbial concentration or infection risk.

Appendix F

Scientific Background

Figure F-1

Jablonski Diagram

Illustration of fluorescence excitation, vibrational relaxation, and emission.

Figure F-2

Electromagnetic Spectrum

Ultraviolet, visible, and infrared regions with highlighted ALS wavelengths.

Figure F-3

Excitation and Emission Spectra of Biological Fluorophores

Including:

- Tryptophan
 - NADH
 - FAD
 - Collagen
 - Elastin
-

Figure F-4

Typical ALS Detection Principle

ALS → Biological Residue → Fluorescence → Barrier Filter → Camera

Appendix G

UVC Safety and Risk Assessment

Hazard	Risk Level	Mitigation
Eye exposure	High	Protective eyewear
Skin exposure	Medium	Shielding
Reflected radiation	Medium	Controlled positioning
Incorrect dose	Medium	Standard operating procedures
Equipment misuse	Low	Operator training

Purpose and Scope

This appendix defines the principal UVC hazards associated with the handheld platform and the engineering, procedural and personal-protective controls required to reduce foreseeable exposure during intended use and reasonably foreseeable misuse. It should be read together with the safety discussion in Chapter 5 and the technical validation requirements in Appendix L.

Safety-Control Architecture

The platform uses a layered safety concept rather than relying on a single protective measure. The precision 5° optical system confines UVC delivery to a defined treatment area; the Orange Halo Distance Guide supports reproducible positioning at the specified 10 cm working distance; and the gyroscope-controlled safety switch interrupts UVC output when an unsafe device orientation is detected. Protective eyewear, operator instructions and defined operating procedures provide additional controls.

Principal Hazards and Required Controls

Direct eye exposure

UVC can cause acute photokeratitis and other ocular injury. The UVC beam must never be directed towards the operator or another person. Gyroscope-controlled interruption is an engineering safeguard and does not replace appropriate protective eyewear, training and controlled handling.

Direct skin exposure

Unnecessary skin exposure must be avoided. The device should be directed only at the intended treatment surface and operated for the validated treatment interval. Hands and other body parts must not enter the treatment beam.

Reflected and scattered UVC

Highly reflective, metallic, glossy or curved surfaces can redirect part of the incident radiation. Operating procedures should therefore require assessment of the treatment

geometry and avoidance of positions that could reflect UVC towards the operator or bystanders.

Incorrect working distance or angle

Delivered irradiance and dose depend on distance, incidence angle and optical geometry. The Orange Halo Distance Guide is intended to support the defined 10 cm working distance. Treatment outside validated geometry can result in under-dosing, altered beam coverage or unintended exposure.

Gyroscope or safety-interlock failure

The automatic UVC interruption function should be treated as a safety-critical control. Its orientation threshold, response time, repeatability, fail-safe behaviour and behaviour under low-battery or fault conditions require documented verification under Appendix L.

Unintended activation and access by bystanders

UVC activation should occur only during deliberate treatment by an authorized operator. The treatment area should be controlled so that bystanders cannot enter the beam path. Storage and transport procedures should prevent unintended activation and unauthorized use.

Personal Protective Equipment and Administrative Controls

Orange UVC-protective inspection glasses supplied or specified for the platform should be used in accordance with the manufacturer's instructions and applicable occupational-safety requirements. PPE is a supplementary control and must not be used as a substitute for engineering safeguards. Operators should receive instruction covering UVC hazards, correct use of the Orange Halo Distance Guide, safe device orientation, reflective surfaces, treatment-time selection, pre-use safety checks and response to equipment faults.

Residual Risk

No handheld high-intensity UVC system can eliminate all exposure risk. Residual risk is controlled through the combined use of precision optics, defined treatment geometry, automatic orientation monitoring, protective eyewear, operator procedures, maintenance and validation. The system should be used only within the validated operating conditions and applicable occupational-safety requirements.

Appendix H

Example Business Case

Example ROI Calculation

Parameter	Conventional Cleaning	ALS/UVC Verification
Repeat cleaning	High	Reduced
ATP consumables	High	Reduced
Inspection documentation	Manual	Automated
Complaint investigations	Reactive	Preventive
Contractor verification	Subjective	Objective

Potential Benefits

- Reduced labour costs
 - Improved cleaning efficiency
 - Better regulatory compliance
 - Enhanced customer confidence
 - Improved contractor performance
 - Objective audit documentation
-

Appendix I

Sample Inspection Report

Inspection Information

Facility:

Location:

Date:

Inspector:

ALS System:

Inspection Results

Surface	Observation	Corrective Action
Flush button	High fluorescence	Cleaned
Faucet	Moderate fluorescence	Cleaned
Door handle	High fluorescence	Cleaned

Verification

☐ No residual fluorescence observed

☐ Additional cleaning required

Inspector Signature

Appendix J

Future Research Roadmap

Short-Term Research (1–2 Years)

- Correlation with ATP measurements
 - Correlation with microbiological cultures
 - Repeatability studies
 - Operator variability
-

Mid-Term Research (3–5 Years)

- AI-assisted fluorescence analysis
 - Automated hotspot detection
 - Quantitative fluorescence scoring
 - Cost-effectiveness studies
 - Multi-centre validation studies
-

Long-Term Research (5–10 Years)

- International inspection standards
 - ISO guideline development
 - Integration into digital facility management systems
 - Predictive hygiene analytics
 - Fully automated fluorescence-based inspection systems
-

Appendix K

Representative UVC Dose Ranges for Surface Decontamination

The values below are representative ranges compiled in the earlier version of this white paper from published scientific literature. Required UVC doses vary with wavelength, microorganism and strain, surface material, organic load, humidity, shadowing, geometry, and the validation method used. The table is therefore a scientific orientation tool, not a product-specific performance claim. Any claimed log reduction must be linked to the delivered dose and a defined test protocol. This appendix supports the dose and log-reduction discussion in Chapter 5 and should be read together with the validation requirements in Appendix L.

Microorganism	Typical Reduction	Approximate Dose (mJ/cm ²)
Escherichia coli	99.9%	5–10
Staphylococcus aureus	99.9%	8–15
MRSA	99.9%	10–20
Salmonella enterica	99.9%	8–15
Pseudomonas aeruginosa	99.9%	10–20
Candida albicans	99.9%	15–30
SARS-CoV-2	>99.9%	3–10
Influenza A	>99.9%	5–10
Human coronavirus 229E	>99.9%	3–8
Norovirus surrogate	>99%	20–40
Clostridioides difficile spores	>99%	80–150

Important considerations

UVC effectiveness depends on delivered dose, not exposure time alone.
Shadowed or recessed areas receive less irradiation than directly exposed surfaces.
Organic residues can absorb or scatter UVC and shield microorganisms.
Organism susceptibility varies substantially; spores generally require higher doses.
Dose tables from the literature cannot substitute for device-specific validation under defined operating conditions.

Dose and exposure time

Delivered dose is calculated from irradiance and exposure time: Dose (mJ/cm²) = Irradiance (mW/cm²) × Exposure Time (s). This relationship allows a validated irradiance at a defined working distance to be translated into a reproducible treatment time. However, a calculated dose alone does not establish a microbiological performance claim; organism- and surface-specific validation remains required.

Appendix L

Scientific Validation Protocol

This protocol restores the quantitative validation framework from Version 1.0 and provides a structured route for converting the operational pilot observations into reproducible scientific evidence. It is designed to evaluate ALS detection performance, cleaning outcomes, and UVC microbial reduction using independent reference methods. It provides the principal route for independently validating product-specific irradiance, treatment-time, safety, and microbial-reduction claims made in Chapters 5 and 10.

L.1 Study Objectives

Determine the relationship between ALS fluorescence findings and established hygiene indicators.

Measure repeatability and reproducibility across operators and facilities.

Quantify the effect of corrective cleaning on fluorescence and ATP measurements.

Quantify microbial reduction following UVC treatment under defined conditions.

Assess whether the complete workflow can be integrated into routine operations without excessive inspection time.

L.2 Recommended Study Design

Use multiple facilities and multiple high-touch surface types to capture real-world variability.

Record each defined surface as one observation and repeat inspections over multiple rounds.

Include representative surfaces such as flush buttons, door handles, faucet controls, soap dispensers, paper towel dispensers, toilet paper holders, grab rails, elevator buttons, touch screens, and payment terminals.

Where feasible, include 500–1,000 individual surface observations. A practical example is 10 facilities × 10 surfaces × 10 inspection rounds = 1,000 observations.

L.3 Standardized Study Workflow

Step	Procedure
1	Baseline white-light and ALS inspection
2	Fluorescence scoring and image documentation
3	Baseline ATP and microbiological sampling
4A	Conventional arm: cleaning → validated UVC dose
4B	Proposed pre-contact arm: targeted UVC dose → cleaning
5	Repeat ALS inspection and image documentation
6	Repeat ATP and microbiological sampling
7	Compare microbial reduction, residue removal, transfer risk, safety, treatment time, and usability between study arms

L.4 Fluorescence Scoring System

Score	Description
0	No detectable fluorescence
1	Very slight localized fluorescence
2	Mild fluorescence
3	Moderate contamination
4	Extensive fluorescence
5	Heavy widespread contamination

Where image-analysis software is available, the study should additionally quantify fluorescent area (%), fluorescence intensity, hotspot count, and contamination distribution. These measurements provide a more objective basis for correlation and trend analysis.

L.5 ATP Validation

ATP samples should be collected immediately adjacent to the photographed ALS inspection area and recorded as Relative Light Units (RLU). Measurements should be taken before and after corrective cleaning; an additional post-UVC ATP measurement may be collected for process documentation, although ATP does not directly measure microbial viability. The primary analysis is the relationship between ALS score and ATP RLU. Perfect agreement is not expected because the methods measure different biological properties.

L.6 Microbiological Validation

Reference microbiological sampling should use standardized contact plates or sterile swabs and accredited laboratory procedures where applicable. Depending on the facility type, outcome measures may include total aerobic count (CFU/cm²), Enterobacteriaceae (CFU/cm²), Staphylococcus aureus presence/absence, and Escherichia coli presence/absence. Additional organisms may be selected according to the intended application.

L.7 UVC Validation and Log Reduction

Microbiological samples should be collected immediately before and after UVC exposure on the selected validation surfaces. The primary outcome is log₁₀ reduction, calculated as log₁₀(Before CFU / After CFU). A reduction of ≥3-log can be used as a practical validation target where operationally achievable and appropriate for the application, but the required target must be defined prospectively for each organism and use case.

L.8 Repeatability and Reproducibility

Intra-operator repeatability: the same operator inspects identical surfaces on three consecutive occasions.

Inter-operator reproducibility: at least three independent operators inspect the same surfaces under standardized settings.

Recommended statistics include coefficient of variation, Cohen's Kappa, Fleiss' Kappa, and Intraclass Correlation Coefficient (ICC), selected according to the measurement scale.

L.9 Statistical Analysis

Analysis objective	Recommended method
ALS vs ATP	Correlation analysis; regression where appropriate
ALS vs microbiology	Correlation plus diagnostic-performance analysis
Diagnostic performance	ROC analysis, sensitivity, specificity, positive predictive value, negative predictive value
Before/after comparisons	Paired t-test or Wilcoxon signed-rank test, depending on data distribution
Operator agreement	Cohen's Kappa, Fleiss' Kappa, ICC
Prediction modelling	Regression analysis for ATP values, CFU counts, and hotspot classification

L.10 Acceptance Criteria

High intra-operator repeatability.
High inter-operator reproducibility.
Statistically significant association between ALS scores and ATP measurements.
Significant reduction in fluorescence following corrective cleaning.
Significant microbial reduction following UVC treatment.
Inspection time compatible with routine operations.
Positive user acceptance by cleaning personnel.

L.11 Quality Assurance and Reporting

All operators should receive standardized training, equipment should be calibrated according to manufacturer recommendations, predefined ALS settings should be used throughout the study, and laboratory analyses should follow accredited procedures such as ISO/IEC 17025 where applicable. Data collection forms should be predefined and original image files archived without alteration. Future studies should use internationally recognized reporting frameworks, including STARD for diagnostic-performance studies, STROBE for observational studies, and relevant CONSORT principles for interventional comparisons where appropriate.

L.12 Expected Scientific Outcome

A successfully completed validation programme will establish whether ALS provides repeatable detection of residual organic contamination, quantify its relationship with ATP and microbiological reference methods, measure the effect of fluorescence-guided corrective cleaning, and determine the microbial reduction delivered by UVC under defined operating conditions. The resulting dataset can support peer-reviewed publication, multicentre validation, regulatory dialogue, standardized operating guidance, and future AI-assisted contamination analysis.

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Author's Recommendation

The validation framework presented in this appendix is intended as a foundation for the systematic scientific evaluation of fluorescence-assisted hygiene verification. While it provides a comprehensive methodological structure, future validation studies should be designed in accordance with internationally recognized research and reporting standards to maximize scientific rigor, reproducibility, and regulatory acceptance.

Where appropriate, diagnostic performance studies should follow the principles of **STARD (Standards for Reporting Diagnostic Accuracy Studies)** to ensure transparent reporting of sensitivity, specificity, predictive values, and overall diagnostic performance. Comparative or interventional investigations should incorporate relevant elements of the **CONSORT** reporting framework, while observational field studies benefit from the **STROBE** guidelines. In addition, laboratory procedures, quality assurance measures, and data management should align with internationally recognized standards such as **ISO 9001**, **ISO/IEC 17025**, and other applicable quality management systems.

Future validation should ideally extend beyond a single institution through multicentre studies involving different facility types, geographic regions, and operational environments. Such studies would enable assessment of repeatability, reproducibility, inter-operator agreement, and the generalizability of fluorescence-assisted inspection under real-world conditions.

The combination of ALS inspection, structured documentation, targeted corrective cleaning, and supplementary UVC decontamination represents a promising quality assurance framework. However, broader scientific acceptance will depend on robust clinical and operational evidence demonstrating reproducibility, correlation with established hygiene indicators, cost-effectiveness, and measurable improvements in cleaning performance.

Successful completion of these validation studies would not only strengthen confidence among researchers, healthcare institutions, regulatory authorities, and industry partners, but also contribute to the development of future international guidelines and standardized protocols for evidence-based hygiene verification.

Ultimately, the objective extends beyond validating a single technology. It is to support the evolution of hygiene management from subjective visual assessment toward a transparent, measurable, and scientifically verified quality assurance process.