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Research article

# Inactivation of *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* using the Contamination Sanitization Inspection and Disinfection (CSI-D) device

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ABSTRACT

The Contamination Sanitization Inspection and Disinfection (CSI-D) device is a handheld fluorescence-based imaging system designed to disinfect food contact surfaces using ultraviolet-C (UVC) illumination. This study aimed to determine the optimal CSI-D parameters (i.e., UVC exposure time and intensity) for the inactivation of the following foodborne bacteria plated on non-selective media: generic *Escherichia coli* (indicator organism) and the pathogens enterohemorrhagic *E. coli*, enterotoxigenic *E. coli*, *Salmonella enterica*, and *Listeria monocytogenes*. Each bacterial strain was spread-plated on non-selective agar and exposed to high-intensity (10 mW/cm$^2$) or low-intensity (5 mW/cm$^2$) UVC for 1–5 s. Control plates were not exposed to UVC. The plates were incubated overnight at 37 °C and then enumerated. Three trials for each bacterial strain were conducted. Statistical analysis was carried out to determine if there were significant differences in bacterial growth between UVC intensities and exposure times. Overall, exposure to low or high intensity for 3–5 s resulted in consistent inhibition of bacterial growth, with reductions of 99.9–100 % for *E. coli*, 96.8–100 % for *S. enterica*, and 99.2–100 % for *L. monocytogenes*. The 1 s exposure time showed inconsistent results, with a 66.0–100 % reduction in growth depending on the intensity and bacterial strain. When the results for all strains within each species were combined, the 3–5 s exposure times showed significantly greater ($p < 0.05$) growth inhibition than the 1 s exposure time. However, there were no significant differences ($p > 0.05$) in growth inhibition between the high and low UVC intensities. The results of this study show that, in pure culture conditions, exposure to UVC with the CSI-D device for ≥3 s is required to achieve consistent reduction of *E. coli, S. enterica,* and *L. monocytogenes*.

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## 1. Introduction

An estimated 48 million cases of foodborne illness occur annually in the United States [1]. Three common pathogenic bacteria known to cause foodborne illness with various health complications are *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes*. While most *E. coli* are harmless, pathogenic strains such as enterohemorrhagic *E. coli* (EHEC) or enterotoxigenic *E. coli* (ETEC) can lead to severe gastrointestinal illness [2]. In the U.S., EHEC accounts for an estimated 265,000 foodborne illnesses annually [2]. ETEC is a major cause of travelers' diarrhea and has been estimated to cause over 24,000 cases annually in North America [3]. *S. enterica* is the leading cause of bacterial foodborne illness in the U.S., with over 1 million estimated cases annually [4]. The top four *S. enterica* serotypes associated with human infection are Enteritidis, Newport, Typhimurium, and Javiana [5]. *L. monocytogenes* causes a relatively small number of cases in the U.S. (~1600 annually); however, over 16 % of cases result in death [6]. The top three *L. monocytogenes* serotypes associated with foodborne illness in the U.S. are 1/2a, 1/2b, and 4b [7].

*E. coli* and *S. enterica* are Gram-negative bacteria commonly associated with fecal-oral transmission, while *L. monocytogenes* is a Gram-positive bacterium widespread in the environment [8]. These bacteria can enter food processing facilities in numerous ways, including via raw materials, contaminated ingredients, pests, and employee clothing or shoes, and can subsequently contaminate food contact surfaces [9,10]. Under ideal conditions of moisture, pH, and temperature, bacterial biofilms may form [11]. These biofilms can attach to surfaces and create a protective barrier, thus making the bacteria more resistant to commonly used cleaning and sanitizing techniques.

Traditional cleaning and sanitizing methods in food processing facilities include using water, chemicals, and sanitizers to remove food particles and prevent the formation of biofilms on food contact surfaces [12]. While traditional cleaning and sanitizing methods are effective, they can lead to further contamination of surfaces if not used properly and are time-consuming. A potential technology that may help address these challenges is the use of ultraviolet-C (UVC) wavelengths [13–16], which may be combined with traditional methods [17]. UV radiation has been approved by the Food and Drug Administration (FDA) to control surface microorganisms on food and food products [18]. A novel approach uses handheld devices that emit UVC wavelength to disinfect surfaces. However, most research efforts in this area have focused on applications in healthcare settings [e.g., [19]], with limited research into the effectiveness of these devices for the disinfection of food contact surfaces [20,21].

The Contamination Sanitization Inspection and Disinfection (CSI-D) device (SafetySpect Inc., Grand Forks, ND) is a new, handheld fluorescence-based imaging device developed to detect microbial contamination and disinfect surfaces rapidly. The CSI-D device contains multiple light-emitting diodes (LEDs) that utilize two fluorescence excitation wavelengths: violet (405 nm) and UVC (275 nm). These lights can detect organic residue on a surface and inactivate pathogens through UVC exposure [21]. Initial research has indicated that the CSI-D device is effective against *Aspergillus fumigatus, Streptococcus pneumoniae,* and the influenza A virus [21]. Additionally, the CSI-D device was reported to inactivate *E. coli*, *S. enterica*, and *L. monocytogenes* on agar plates following 3–5 s exposure to UVC at intensities of 5–10 mW/cm$^2$ [20]. While Gorji et al. [20] showed promising results for the inactivation of foodborne bacteria with the CSI-D device, the study was limited to a single trial and did not include statistical analysis.

The goal of the current study was to determine the optimal parameters (i.e., UVC exposure time and intensity) for the use of the CSI-D device in the inactivation of various strains of *E. coli*, *S. enterica*, and *L. monocytogenes* plated on non-selective media. This research builds upon the results of Gorji et al. [20] by testing additional bacterial strains, conducting multiple trials per strain, and applying statistical analysis to determine the optimal parameters for inactivation. The targeted bacteria were generic *E. coli*; EHEC; ETEC; *S. enterica* serotypes Enteritidis, Typhimurium, Newport, and Javiana; and *L. monocytogenes* serotypes 1/2a, 1/2b, and 4b.

## 2. Materials and methods

### 2.1. Bacteria and media

The following strains were obtained from the American Type Culture Collection (ATCC, Manassas, VA): generic *E. coli* (ATCC 51813), EHEC (*E. coli* O157:H7; ATCC 35150), ETEC (*E. coli* O78:H11; ATCC 35401), *S. enterica* Javiana (ATCC 10721), *L. monocytogenes* 1/2b (ATCC 51780) and *L. monocytogenes* 4b (ATCC 19115). Environmental isolates of *S. enterica* Enteritidis (PFGE2811), *S. enterica* Newport (FDA 2245), *S. enterica* Typhimurium (FDA 2112), and *L. monocytogenes* 1/2a (FDA 2109) were obtained from the Food and Drug Administration, Irvine Human and Animal Food Laboratory (Irvine, CA). Unless otherwise stated, all media was obtained from Neogen (Lansing, MI, USA).

### 2.2. Bacterial culture methods

The bacterial culture methods used in this study were adapted from Gorji et al. [20], with the specific details for each bacterial strain provided in the subsections below. First, bacterial strains were cultured on selective agar for confirmation of species. Next, an isolated colony was incubated in a non-selective broth (Lennox LB broth for *E. coli* and *S. enterica* and Tryptic Soy Broth for L. *monocytogenes*), followed by serial dilution and spread-plating on L-agar (Lennox LB broth base with 1.5 % agar) prior to exposure to UVC (described in section 2.4). For each dilution, a total of four spread plates were prepared per UVC intensity-time combination, and an additional set of four control plates were prepared that were not exposed to the CSI-D device. Control plates with countable colonies and the corresponding treatment plates were examined to determine differences in colony counts due to treatment (Supplementary Material).

### 2.2.1. Escherichia coli

Frozen cultures of generic *E. coli* and EHEC were streaked onto MacConkey (MAC) agar for isolation, and ETEC was streaked onto colonization factor antigen (CFA) agar [22] for isolation. The plates were incubated at 37 °C for 18–24 h. After incubation, an isolated colony of each generic *E. coli* and EHEC culture was streaked onto MAC agar, MAC agar with sorbitol (SMAC), and Simmons Citrate agar for species confirmation. An isolated colony of ETEC was streaked onto CFA agar, MAC agar, SMAC agar, and Simmons Citrate agar for species confirmation. Isolated colonies of each strain were also streaked on L-agar. All plates were incubated at 37 °C for 18–24 h, then examined for typical colonies associated with the target bacterial strain. An isolated colony of each strain was transferred from L-agar to Luria Bertani (LB) broth for incubation at 37 °C for 18–24 h. Next, serial dilutions were prepared using Phosphate Buffered Saline (PBS, pH 7.4; Fisher Scientific). A 100 μl aliquot of the $10^{-5}$ and $10^{-6}$ dilutions (generic *E. coli*) or the $10^{-6}$ and $10^{-7}$ dilutions (EHEC and ETEC) was spread-plated onto L-agar. Based on the control plates, the concentrations of the pre-treatment microbial cultures ranged from 2.8 to 2.9 log CFU/ml (Table 1).

### 2.2.2. Salmonella enterica

Frozen cultures of *S. enterica* strains were streaked onto XLT4 agar (MilliporeSigma, Burlington, MA, USA) for isolation and incubated at 37 °C for 18–24 h. After incubation, an isolated colony was streaked onto XLT4 and Brilliant Green agar for species confirmation; each strain was also streaked to L-agar. All plates were incubated at 37 °C for 18–24 h, then examined for typical colonies associated with the target bacterial strain. An isolated colony of each strain was transferred from L-agar to Luria Bertani (LB) broth for incubation at 37 °C for 18–24 h. Next, serial dilutions were prepared using PBS. A 100 μl aliquot of the $10^{-6}$ and $10^{-7}$ dilutions (serotypes Typhimurium and Javiana) or of the $10^{-7}$ and $10^{-8}$ dilutions (serotypes Enteritidis and Newport) was spread-plated onto L-agar. Based on the control plates, the concentrations of the pre-treatment microbial cultures ranged from 1.6 to 2.9 log CFU/ml (Table 2).

### 2.2.3. Listeria monocytogenes

Frozen cultures of *L. monocytogenes* ½a, ½b, and 4b were streaked for isolation onto Modified Oxford agar (MOX) and incubated at 37 °C for 24–48 h. After incubation, an isolated colony was streaked onto MOX, Palcam (Oxoid, Lenexa, KS, USA), and Harlequin

**Table 1**
Effects of UVC exposure with the CSI-D device on generic *E. coli*, enterohemorrhagic *E. coli* (EHEC; *E. coli* O157:H7), and enterotoxigenic *E. coli* (ETEC; *E. coli* O78:H11). The results represent the combined data from three trials.

| Bacteria | UVC Intensity | Exposure Time | Average Colony Count (log CFU/ml) | Reduction in Growth Compared to Control[a] | | |
|---|---|---|---|---|---|---|
| | | | | Log Reduction (log CFU/ml) | Percent Reduction (%) | Percent Survival (%) |
| **Generic *E. coli*** | None (Control) | None (Control) | 2.9 | N/A | N/A | NA |
| **Generic *E. coli*** | High | 1 | 0.7 | 2.2[a] | 99.3[a] | 0.7 |
| **Generic *E. coli*** | High | 3 | 0.1 | 2.8[b] | 99.9[b] | 0.1 |
| **Generic *E. coli*** | High | 5 | 0.0 | 2.9[b] | 100.0[b] | 0.0 |
| **Generic *E. coli*** | Low | 1 | 1.6 | 1.3[a] | 94.3[a] | 5.7 |
| **Generic *E. coli*** | Low | 3 | 0.0 | 2.9[b] | 100.0[b] | 0.0 |
| **Generic *E. coli*** | Low | 5 | 0.0 | 2.9[b] | 100.0[b] | 0.0 |
| **EHEC** | None (Control) | None (Control) | 2.9 | N/A | N/A | NA |
| **EHEC** | High | 1 | 0.0 | 2.9[a] | 100.0[a] | 0.0 |
| **EHEC** | High | 3 | 0.0 | 2.9[a] | 100.0[a] | 0.0 |
| **EHEC** | High | 5 | 0.0 | 2.9[a] | 100.0[a] | 0.0 |
| **EHEC** | Low | 1 | 1.5 | 1.3[a] | 94.2[a] | 0.3 |
| **EHEC** | Low | 3 | 0.0 | 2.9[a] | 100.0[a] | 0.0 |
| **EHEC** | Low | 5 | 0.0 | 2.9[a] | 100.0[a] | 0.0 |
| **ETEC** | None (Control) | None (Control) | 2.8 | N/A | N/A | NA |
| **ETEC** | High | 1 | 0.0 | 2.8[a] | 100.0[a] | 0.0 |
| **ETEC** | High | 3 | 0.0 | 2.8[a] | 100.0[a] | 0.0 |
| **ETEC** | High | 5 | 0.0 | 2.8[a] | 100.0[a] | 0.0 |
| **ETEC** | Low | 1 | 0.3 | 2.5[a] | 99.7[a] | 5.8 |
| **ETEC** | Low | 3 | 0.0 | 2.8[a] | 100.0[a] | 0.0 |
| **ETEC** | Low | 5 | 0.0 | 2.8[a] | 100.0[a] | 0.0 |

[ab]A different superscript letter in the same column for the same strain indicates a significant difference based on exposure time, according to the Kruskal-Wallis test with the Dunn's post-hoc test ($p < 0.05$). There were no significant differences based on intensity levels.

[a] Slight discrepancies in the values across columns are due to rounding.

**Table 2**
Effects of UVC exposure with the CSI-D device on *S. enterica* Enteritidis, Newport, Typhimurium, and Javiana following UVC exposure with the CSI-D device. The results represent the combined data from three trials.

| *Salmonella* Serotype | UVC Intensity | Exposure Time | Average Colony Count (log CFU/ml) | Reduction in Growth Compared to Control[a] | | |
|---|---|---|---|---|---|---|
| | | | | Log Reduction (log CFU/ml | Percent Reduction (%) | Percent Survival (%) |
| **Enteritidis** | None (Control) | None (Control) | 2.1 | N/A | N/A | N/A |
| **Enteritidis** | High | 1 | 0.3 | 1.9[a] | 99.4[a] | 0.6 |
| **Enteritidis** | High | 3 | 0.0 | 2.1[a] | 100.0[a] | 0.0 |
| **Enteritidis** | High | 5 | 0.0 | 2.1[a] | 100.0[a] | 0.0 |
| **Enteritidis** | Low | 1 | 0.5 | 1.6[a] | 93.4[a] | 6.6 |
| **Enteritidis** | Low | 3 | 0.3 | 1.9[a] | 96.8[a] | 3.2 |
| **Enteritidis** | Low | 5 | 0.1 | 2.0[a] | 99.8[a] | 0.2 |
| **Newport** | None (Control) | None (Control) | 1.6 | N/A | N/A | N/A |
| **Newport** | High | 1 | 0.1 | 1.4[a] | 98.8[a] | 1.2 |
| **Newport** | High | 3 | 0.2 | 1.3[a] | 97.5[a] | 2.5 |
| **Newport** | High | 5 | 0.0 | 1.6[a] | 100.0[a] | 0.0 |
| **Newport** | Low | 1 | 0.4 | 1.1[a] | 94.8[a] | 5.2 |
| **Newport** | Low | 3 | 0.1 | 1.4[a] | 98.8[a] | 1.2 |
| **Newport** | Low | 5 | 0.0 | 1.6[a] | 100.0[a] | 0.0 |
| **Typhimurium** | None (Control) | None (Control) | 2.9 | N/A | N/A | N/A |
| **Typhimurium** | High | 1 | 0.4 | 2.5[a] | 99.3[a] | 0.7 |
| **Typhimurium** | High | 3 | 0.0 | 2.9[a] | 100.0[b] | 0.0 |
| **Typhimurium** | High | 5 | 0.1 | 2.7[a] | 99.7[b] | 0.3 |
| **Typhimurium** | Low | 1 | 1.3 | 1.5[a] | 97.1[a] | 2.9 |
| **Typhimurium** | Low | 3 | 0.1 | 2.7[a] | 99.9[b] | 0.1 |
| **Typhimurium** | Low | 5 | 0.0 | 2.9[a] | 100.0[b] | 0.0 |
| **Javiana** | None (Control) | None (Control) | 2.6 | N/A | N/A | N/A |
| **Javiana** | High | 1 | 0.4 | 2.2[a] | 99.6[a] | 0.4 |
| **Javiana** | High | 3 | 0.0 | 2.6[b] | 100.0[b] | 0.0 |
| **Javiana** | High | 5 | 0.0 | 2.6[b] | 100.0[b] | 0.0 |
| **Javiana** | Low | 1 | 1.0 | 1.6[a] | 94.1[a] | 5.9 |
| **Javiana** | Low | 3 | 0.0 | 2.6[b] | 100.0[b] | 0.0 |
| **Javiana** | Low | 5 | 0.0 | 2.6[b] | 100.0[b] | 0.0 |

[ab]A different superscript letter in the same column for the same strain indicates a significant difference based on exposure time, according to the Kruskal-Wallis test with the Dunn's post-hoc test ($p < 0.05$). There were no significant differences based on intensity levels.
[a] Slight discrepancies in the values across columns are due to rounding.

*Listeria* chromogenic agar (LCA) for species confirmation. An isolated colony was also streaked onto tryptic soy agar with yeast extract (TSA-YE). All plates were incubated at 37 °C for 18–24 h, then examined for typical colonies associated with the target bacterial strain. An isolated colony was transferred from TSA-YE to tryptic soy broth (TSB) for incubation at 37 °C for 18–24 h. Next, serial dilutions were prepared using PBS. A 100 μl aliquot of the $10^{-5}$ and $10^{-6}$ dilutions was spread-plated onto L-agar. Based on the control plates, the concentrations of the pre-treatment microbial populations ranged from 2.6 to 3.0 log CFU/ml (Table 3).

### 2.3. UVC exposure with the CSI-D device

Within 1.5 h of preparing the bacterial dilutions, the spread plates containing each bacterial strain were exposed to UVC with the CSI-D device (Fig. 1) (provided by SafetySpect Inc., North Dakota, USA) at the time and intensity treatment levels described in section 2.2. All UVC treatments were carried out at room temperature. Before beginning the experimental trials, the CSI-D was calibrated for intensity across the field of view, and the spectrum was measured to ensure excitation matched specifications. UVC intensity was measured with a UV radiometer (Radiometer RMD Pro, Opsytec Dr. Gröbel GmbH, Ettingen, Germany). Inoculated agar plates were placed on a platform at a set distance to obtain the high or low UVC intensity measurement. The SafetySpect, Inc. graphical user interface (GUI) was used to control the device, with a built-in timer to allow for precise UVC exposure for 1, 3, or 5 s. Exposed and control plates were incubated at room temperature (20–22 °C) for 2–3 h, then at 37 °C for 18–20 h (*E. coli* and *S. enterica*) or 24–48 h (*L. monocytogenes*). Following incubation, the colonies on each plate were enumerated, and the average colony forming units (CFUs) per ml was determined.

### 2.4. Experimental design and data analysis

Each bacterial strain was tested using a 2 × 3 factorial treatment arrangement with exposure of bacteria to the CSI-D at two levels of UVC intensity (low = 5 mW/cm$^2$ and high = 10 mW/cm$^2$) and three levels of time (1, 3, and 5 s), for a total of six total treatments [20].

**Table 3**
Effects of UVC exposure with the CSI-D device on *L. monocytogenes* 1/2a, 1/2b, and 4b following UVC exposure with the CSI-D device. The results represent the combined data from three trials.

| *Listeria* Serotype | UVC Intensity | Exposure Time | Average Colony Count (log CFU/ml) | Reduction in Growth Compared to Control[a] | | |
|---|---|---|---|---|---|---|
| | | | | Log Reduction (log CFU/ml) | Percent Reduction (%) | Percent Survival (%) |
| **1/2a** | None (Control) | None (Control) | 2.6 | N/A | N/A | N/A |
| **1/2a** | High | 1 | 1.6 | 1.0[a] | 89.9[a] | 10.1 |
| **1/2a** | High | 3 | 0.1 | 2.5[b] | 99.9[b] | 0.1 |
| **1/2a** | High | 5 | 0.0 | 2.6[b] | 100.0[b] | 0.0 |
| **1/2a** | Low | 1 | 2.1 | 0.5[a] | 66.0[a] | 34.0 |
| **1/2a** | Low | 3 | 0.5 | 2.1[b] | 99.2[b] | 0.8 |
| **1/2a** | Low | 5 | 0.0 | 2.6[b] | 100.0[b] | 0.0 |
| **1/2b** | None (Control) | None (Control) | 3.0 | N/A | N/A | N/A |
| **1/2b** | High | 1 | 1.3 | 1.7[a] | 98.0[a] | 2.0 |
| **1/2b** | High | 3 | 0.0 | 3.0[b] | 100.0[b] | 0.0 |
| **1/2b** | High | 5 | 0.0 | 3.0[b] | 100.0[b] | 0.0 |
| **1/2b** | Low | 1 | 2.2 | 0.8[a] | 76.8[a] | 23.2 |
| **1/2b** | Low | 3 | 0.4 | 2.6[b] | 99.7[b] | 0.3 |
| **1/2b** | Low | 5 | 0.0 | 3.0[b] | 100.0[b] | 0.0 |
| **4b** | None (Control) | None (Control) | 2.8 | N/A | N/A | N/A |
| **4b** | High | 1 | 1.3 | 1.6[a] | 97.0[a] | 3.0 |
| **4b** | High | 3 | 0.0 | 2.8[b] | 100.0[b] | 0.0 |
| **4b** | High | 5 | 0.0 | 2.8[b] | 100.0[b] | 0.0 |
| **4b** | Low | 1 | 1.9 | 1.0[a] | 89.2[a] | 10.8 |
| **4b** | Low | 3 | 0.0 | 2.8[b] | 100.0[b] | 0.0 |
| **4b** | Low | 5 | 0.0 | 2.8[b] | 100.0[b] | 0.0 |

[ab]A different superscript letter in the same column for the same strain indicates a significant difference based on exposure time, according to the Kruskal-Wallis test with the Dunn's post-hoc test ($p < 0.05$). There were no significant differences based on intensity levels.
[a] Slight discrepancies in the values across columns are due to rounding.

**Fig. 1.** Image of the CSI-D device.

The treatments were repeated across three trials on three separate days, with four agar plates per treatment, resulting in 18 treatment samples (72 agar plates) per bacterial strain (Supplementary Material). Microbial growth on the UVC-exposed plates was compared to the control plates to determine the reduction in bacterial growth due to UVC treatment. Log reduction and percent reduction were calculated using the following formulas: $Log\ Reduction = \log_{10}(A) - \log_{10}(B)$ and $Percent\ Reduction = \frac{(A-B)\ x\ 100}{A}$, where A is the average CFU/ml before treatment (i.e., control) and B is the average CFU/ml after treatment (Supplementary Material). Samples with no visible colonies on all four treatment plates from a given trial were assigned an average value of 1 CFU/ml (0 log CFU/ml) for the purpose of calculating log reductions. The limit of detection (LOD) for the bacterial counts was determined to be one colony across the

four treatment plates, corresponding to 2.5 CFU/ml or 0.4 log CFU/ml.

The data was statistically analyzed based on UVC intensity and exposure time, using two levels for UVC intensity (low and high) and three levels for exposure time (1, 3, and 5 s). The mean and standard deviation for all combinations of variables were calculated. Normality and homoscedasticity were determined using the Shapiro-Wilk normality test and Bartlett test of homogeneity of variances. Data was analyzed using the Kruskal-Wallis one-way analysis of variance by ranks. All p-values were compared using a significance level of $\alpha = 0.05$. Statistically significant results were compared using Dunn's post-hoc test with Bonferroni correction. All statistical analysis described above was performed using RStudio version 4.2.1 [23]. Linear regression analysis was carried out in Excel (Microsoft Office, Redmond, WA, USA), with the independent variable being time or intensity and the dependent variable being log reduction (CFU/ml).

## 3. Results and discussion

### 3.1. E. coli

The average of the three trials for generic *E. coli*, EHEC, and ETEC showed that an exposure time of at least 3 s at either high or low UVC intensity resulted in consistent reduction of 99.9–100 % for all three *E. coli* strains tested (Table 1). At the 1 s exposure time, there was a 94.2–99.7 % reduction in bacterial growth at low UVC intensity and a 99.3–100 % reduction at high UVC intensity. Generic *E. coli* showed a 100 % reduction in bacterial growth following exposure to either high or low UVC intensity for 5 s or low UVC intensity for 3 s, with no detectable growth following treatment. These results were consistent with Gorji et al. [20], who reported no detectable growth for generic *E. coli* at both UVC intensities following 3–5 s exposure; however, the authors also found no detectable growth for both UVC intensities following 1 s exposure. In the current study, the 1 s exposure time showed detectable growth of generic *E. coli* following treatment, with survival rates of 0.7–5.7 % (Table 1).

A 100 % reduction in growth of EHEC and ETEC was observed when plates were treated with the CSI-D device for 3–5 s at either UVC intensity level or for 1 s at high UVC intensity (Table 1), with no detectable growth following treatment. These results were similar to Gorji et al. [20], who reported no detectable growth of EHEC following 1–5 s exposure to low or high UVC intensity. However, in the current study, survival rates of EHEC and ETEC were 0.3–5.8 % following 1 s exposure at low UVC intensity (Table 1). The variation in results at the 1 s exposure time indicates that the device is less effective when very short exposure times are employed; therefore, 3–5 s exposure times are recommended. In comparison, previous studies using slightly different experimental conditions have also reported UVC-LED treatment to be effective against *E. coli,* with one study reporting up to a 99.999 % reduction in EHEC following exposure to 2 $mJ/cm^2$ [14] and another study reporting up to a 99.9 % reduction in *E. coli* following exposure to 6750 $\mu J/cm^2$ [15].

Based on the combined data for all three *E. coli* strains, the 3–5 s exposure times resulted in significantly greater ($p < 0.05$) reductions in bacterial growth (i.e., log reduction and percent reduction) than the 1 s exposure time, according to the Kruskal-Wallis test with the Dunn's post-hoc test. There were no significant differences ($p > 0.05$) between the 3 and 5 s exposure times. When the strains were analyzed individually, this trend was also observed for generic *E. coli* (log reduction and percent reduction) (Table 1). However, there were no significant differences ($p > 0.05$) observed for ETEC or EHEC across the different exposure times. When comparing the results across the low and high UVC intensities, there were no significant differences ($p > 0.05$) in the percent reduction or log reduction values. These findings indicate that the CSI-D device is significantly more effective at inactivating *E. coli* at extended exposure times. The results of linear regression analysis on all *E. coli* strains combined showed a weak correlation ($R^2 = 0.33$) between time and log reduction and no correlation ($R^2 = 0.09$) between intensity and log reduction (Supplementary Material).

Overall, there was a consistent reduction in growth of generic *E. coli*, EHEC, and ETEC following treatment with the CSI-D device for 3–5 s. The high susceptibility of *E. coli* to the CSI-D device is likely due to the effectiveness of UVC wavelengths on Gram-negative bacteria [24]. While the results for generic *E. coli* and EHEC are consistent with the results of Gorji et al. [20] at the higher exposure times, this is the first report of the effectiveness of the CSI-D device against ETEC.

### 3.2. S. enterica

The average of the three trials for *S. enterica* serotypes Enteritidis, Newport, Typhimurium, and Javiana showed that an exposure time of at least 5 s at either high or low UVC intensity resulted in a consistent reduction of 99.7–100 % of *S. enterica* (Table 2). The 3 s exposure time resulted in a 96.8–100 % reduction in bacterial growth at either high or low intensity. At the 1 s exposure time, 93.4–100 % reduction levels were observed at low UVC intensity, and 98.8–99.6 % reduction levels were observed at high UVC intensity.

As shown in Table 2, there was a 100 % reduction in bacterial growth for *S. enterica* serotypes Enteriditis and Javiana when exposed to high UVC intensity for 3–5 s, with no detectable growth following treatment. There was also a 100 % reduction in growth of *S. enterica* Javiana following exposure to low UVC intensity for 3–5 s, whereas the reduction in *S. enterica* Enteriditis growth was 96.8–99.8 % after exposure to low UVC intensity for 3–5 s. Reductions in growth for serotypes Newport and Typhimurium were 97.5–100 % (3 s exposure) and 99.7–100 % (5 s exposure) at either UVC intensity. In comparison, Gorji et al. [20] reported no detectable growth of *S. enterica* Enteritidis and Typhimurium following 3–5 s exposure to either low or high UVC intensity. Related studies have also shown UVC-LED treatment to be effective against *Salmonella,* with reports of 99–99.99 % reduction in *Salmonella* Typhimurium following exposure to 5–6.75 $mJ/cm^2$ [14,15] and 87.7–99.999 % reduction for a five-strain cocktail of *S. enterica* on food contact surfaces following exposure to 2–4 $mW/cm^2$ [13].

In agreement with the findings for *E. coli*, a 1 s exposure time was less effective at reducing growth of *S. enterica* than the 3–5 s

exposure times. At both UVC intensities, there was detectable bacterial growth following 1 s exposure for all four serotypes tested, with survival rates of 0.4–6.6 % (Table 2). Similarly, Gorji et al. [20] reported detectable growth of *S. enterica* Typhimurium when exposed to low UVC intensity for 1 s; however, no detectable growth was observed following exposure to high UVC intensity for 1 s.

Based on the combined data for all four *Salmonella* strains, the 3–5 s exposure times resulted in significantly greater ($p < 0.05$) reductions in bacterial growth (i.e., log reduction and percent reduction) than the 1 s exposure time, according to the Kruskal-Wallis test with the Dunn's post-hoc test. There were no significant differences ($p > 0.05$) between the 3 and 5 s exposure times. When the strains were analyzed individually, this trend continued to be observed for serotypes Typhimurium (percent reduction only) and Javiana (log reduction and percent reduction) (Table 2). However, there were no significant differences ($p > 0.05$) observed for serotypes Enteritidis and Newport across the different exposure times. When comparing the results across the low and high UVC intensities, there were no significant differences ($p > 0.05$) in the percent reduction or log reduction values for any of the strains analyzed. The results of linear regression analysis on all *Salmonella* strains combined showed little to no correlation ($R^2 = 0.15$) between time and log reduction and no correlation ($R^2 = 0.03$) between intensity and log reduction (Supplementary Material).

The reduction in *S. enterica* growth following UVC exposure for 3–5 s is likely due to the effectiveness of UVC wavelengths on Gram-negative bacteria [24]. While the results of *S. enterica* Enteritidis and Typhimurium are consistent with the results of previous studies, this is the first report of the effectiveness of the CSI-D device against serotypes Newport and Javiana.

### 3.3. *L. monocytogenes*

The average of the three trials for *L. monocytogenes* serotypes 1/2a, 1/2b, and 4b showed that an exposure time of at least 3 s at either high or low UVC intensity resulted in reduction in bacterial growth at rates of 99.2–100 % (Table 3). Growth of L. *monocytogenes* was consistently 100 % inhibited following an exposure time of 5 s and by ≥ 99.2 % following an exposure time of 3 s. In comparison, Gorji et al. [20] reported no detectable growth following 3–5 s exposure to low or high UVC intensity for *L. monocytogenes* serotypes 1/2a, 1/2b, and 4b.

In line with the results found for *E. coli* and *S. enterica*, the results for *L. monocytogenes* showed that the CSI-D device is less effective at reducing bacterial growth at the 1 s exposure time with low UVC intensity (Table 3). Specifically, survival rates of 10.8–34.0 % and 2.0–10.1 % were observed following 1 s exposure at low and high UVC intensity, respectively. These results are similar to those of Gorji et al. [20], who reported detectable bacterial growth at the 1 s exposure time with low UVC intensity for the same three serotypes. However, Gorji et al. [20] reported no detectable growth for most serotypes treated at the 1 s exposure time with high UVC intensity. Similarly, Kim and Kang [14] reported a 99–99.9 % reduction in *L. monocytogenes* following exposure to UVC-LEDs at 5 mJ/cm$^2$.

When the data for all three *Listeria* strains was combined, the 3–5 s exposure times resulted in significantly greater ($p < 0.05$) reductions in bacterial growth (i.e., log reduction and percent reduction) than the 1 s exposure time, according to the Kruskal-Wallis test with the Dunn's post-hoc test. There were no significant differences ($p > 0.05$) between the 3 and 5 s exposure times. This trend was also observed for all three serotypes when the strains were analyzed individually (Table 2). When the results were compared across the low and high UVC intensities, no significant differences ($p > 0.05$) were observed in the percent reduction or log reduction values for any of the strains analyzed. Along these lines, the results of linear regression analysis on all *Listeria* strains combined showed a strong correlation ($R^2 = 0.72$) between time and log reduction and no correlation ($R^2 = 0.03$) between intensity and log reduction (Supplementary Material).

Overall, the growth of *L. monocytogenes* serotypes 1/2a, 1/2b, and 4b was consistently reduced following treatment with the CSI-D device for 3–5 s with low or high UVC intensity. However, when compared with *E. coli* and *S. enterica,* the CSI-D device was less effective at reducing the growth of *L. monocytogenes* following the 1 s exposure time. The enhanced resistance of *L. monocytogenes* to the CSI-D device may be attributed to previous findings that a higher UVC intensity is required to penetrate the cell wall of Gram-positive bacteria [24]. Similarly, Kim and Kang [14] also found reduced effectiveness for UVC-LED treatment against *L. monocytogenes* compared with *E. coli* and *S. enterica*.

## 4. Conclusion

This study determined the optimal parameters for using the CSI-D device to inactivate foodborne bacteria, including strains of pathogenic *E. coli*, *S. enterica*, and *L. monocytogenes* in pure culture. Overall, 3–5 s exposure times at low (5 mW/cm$^2$) or high (10 mW/cm$^2$) intensity were required to consistently inhibit bacterial growth. These conditions allowed for a 96.8–100 % reduction in bacterial growth for *E. coli, S. enterica, and L. monocytogenes*. On the other hand, a 1 s exposure time resulted in inconsistent bacterial inactivation, with growth reduction rates of 60.0–100 % across the various strains tested. The Gram-positive *L. monocytogenes* was generally more resistant to UVC than the Gram-negative bacteria, *S. enterica* and *E. coli,* when treated with the shortest exposure time. It is important to note that these parameters were determined under laboratory conditions where bacterial growth was controlled, organic matter interference was not assessed, and background microflora was absent. Further research is needed to determine if the parameters found to be effective in this study can be applied to inactivate bacteria on food contact surfaces. Overall, the results of this study will inform future use of the CSI-D device to ensure effectiveness in disinfecting food contact surfaces in production facilities, with the ultimate goal of improving the safety of the food supply.

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## Data availability

The data associated with this study has not been deposited into a publicly available repository. The data is included in supplementary material.

## CRediT authorship contribution statement

**Jennifer McCoy Sanders:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vanessa Alarcon:** Writing – review & editing, Investigation. **Grace Marquis:** Methodology, Conceptualization. **Amanda Tabb:** Methodology, Conceptualization. **Jo Ann Van Kessel:** Writing – review & editing, Methodology, Conceptualization. **Jakeitha Sonnier:** Writing – review & editing, Methodology. **Bradd J. Haley:** Writing – review & editing, Methodology. **Insuck Baek:** Methodology. **Jianwei Qin:** Methodology, Conceptualization. **Moon Kim:** Supervision, Methodology, Conceptualization. **Fartash Vasefi:** Software, Methodology. **Stanislav Sokolov:** Software, Methodology. **Rosalee S. Hellberg:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Authors FV and SS own stocks or stock options in SafetySpect Inc. FV is an inventor on US patent application US20210228757A1. The remaining authors declare no conflict of interest.

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## Appendix A. Supplementary data

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