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PEER-REVIEWED ARTICLE

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Adenosine Triphosphate Bioluminescence is a Poor Indicator of *Listeria* spp. Presence in Distribution Centers Handling Fresh Produce

ABSTRACT

Monitoring for residual ATP is a rapid method used throughout the food industry to verify the efficacy of cleaning procedures for removing organic material prior to sanitation. Efforts to use ATP readings as a predictor of foodborne pathogens within the food environment were examined with mixed results. Therefore, ATP was investigated as an indicator for the presence of *Listeria* species in 17 U.S. food distribution center environments. Environmental surface samples (n = 300) were collected concurrently with ATP swabs to determine *Listeria* spp. presence and ATP relative light units (RLU) at given sampling sites. The number of *Listeria* spp.-positive samples were 13 (4.3%) of 300. ATP RLU varied widely across individual distribution centers, with an average of ca. 559 RLU and an overall range of ca. 0 to 8,690 RLU. Logistic regression to predict *Listeria* spp.-positive sample versus log(x + 1) transformed RLU data provided an odds ratio of 0.34; this indicates that ATP bioluminescence was a poor predictor of *Listeria* species presence in the sampled distribution centers. Although ATP does not appear to be a predictor of *Listeria* spp., it still may be an important tool to monitor and verify the efficacy of sanitation programs.

INTRODUCTION

The implementation of the Food Safety Modernization Act has resulted in a significant shift in the manner food safety is addressed in the United States. Most notably, the previously reactive regulatory system to handle foodborne illness outbreaks and recalls transitioned to a more preventive approach *(11)*. Before the Food Safety Modernization Act, several industries were subjected to food safety systems, such as good manufacturing practices *(13)*. However, after Food Safety Modernization Act implementation and the Preventive Controls for Human Food Rule, all FDA-regulated facilities that manufacture, process, pack, or hold any human food intended for consumption in the United States are required to implement a risk-based food safety plan. A comprehensive Preventive Controls for Human Food Rule food safety plan is developed to identify potential hazards and corresponding preventive controls or prerequisite programs (21 C.F.R. § 117.126).

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For instance, a facility identifying a pathogen, such as *Listeria monocytogenes*, as a potential hazard in ready-to-eat food must implement sanitation preventive controls. Efficacy of cleaning and sanitizing protocols (sanitation) requires a verification process, such as an environmental monitoring program *(4, 24)*. There are several environmental monitoring program methods and tools to assist facilities in verifying if sanitation protocols are effective, including an in-house or third-party laboratory to perform microbial swabbing for aerobic plate counts, yeasts, molds, *Enterobacteriaceae*, *Escherichia coli*, and *Listeria* species (or specifically targeting the pathogen *L. monocytogenes*). In addition, rapid methods and tools may also be used as means to verify sanitation, such as the detection of ATP, allergens, and protein and carbohydrate residues.

Specifically, ATP is a molecule that drives cellular function and is found in all biological materials, including cell-containing food matter. Rapid quantification of ATP is possible by using a bioluminescence assay. This assay consists of the consumption of ATP in a reaction between luciferin and luciferase, which produces a fluorescent light *(9)*. The light intensity from this reaction can be quantified by using an ATP luminometer, which estimates ATP in relative light units (RLU) *(5)*. RLU levels are readily displayed on the luminometer in as little as 15 s, providing efficient and rapid results. RLU levels above facility-set thresholds indicate that a surface may not have been adequately cleaned, as biological material is still present on the surface. These set thresholds may vary between the type of food facility, type of surface, and cleaning and sanitizing protocols. For these reasons, it can be challenging to extrapolate ATP data across food facilities, especially in terms of microbial risk.

Given the relative convenience, cost efficiency, quick results, and usefulness in evaluating the cleanliness of surfaces, it is not surprising that ATP assays are frequently used in food facilities, despite disparate evidence regarding the ability to predict the presence of microbial hazards *(14, 18, 20, 21)*. A laboratory-based surface study performed by Lane et al. *(18)* concluded that although ATP bioluminescence could be used to measure *Listeria innocua* on stainless steel and high-density polyethylene coupons, it was unreliable on wood surface coupons. ATP bioluminescence was also compared against aerobic plate counts to evaluate the sanitation of cutting boards *(20)*. Both ATP and aerobic plate counts were comparable when evaluating the sanitation efficacy on cutting boards with bacterial inocula *(20)*. In a preoperational survey of 30 retail delis, Hammons et al. *(14)* found a fourfold increase in the likelihood of detecting *L. monocytogenes* when ATP measurements increased by 1 log RLU on corresponding surfaces. Conversely, a different study found no statistical difference in ATP RLU levels between *Listeria* spp.-positive and-negative food contact surfaces in five commercial apple packinghouses *(21)*. Therefore, the usefulness of ATP is goal specific, as data describing ATP as an indicator of microorganisms vary on the basis of facility, surface, and process, among other factors *(1)*. In addition, the use of ATP is facilitated by an understanding of a cleanliness baseline before and after cleaning activities.

An under-investigated area along the food supply chain is at the transportation and distribution center level (often described as the area between packing and processing and retail) *(30)*. Distribution centers fall under the Preventive Controls for Human Food Rule, as they hold human food for consumption and thus must develop and implement a risk-based food safety plan. Although limited data on microbial hazards in this environment exist, there is concern regarding the possible introduction of environmental pathogens to products in distribution centers *(31)*. Many areas within distribution centers are held at refrigeration temperatures to maintain food quality and safety during storage, and prior research has shown *Listeria* survival and persistence in these temperatures and environments *(7, 29, 31)*. To generate data in this environment and address this concern, a microbial survey was conducted in 17 food distribution centers to quantify *Listeria* spp. on non-food-contact surfaces. In addition, other attribute data (e.g., ATP, sanitation protocol frequency) were collected to investigate associations with *Listeria (31)*. This study used *Listeria* spp. as an index organism for *L. monocytogenes* due to the sensitive nature of detecting an *L. monocytogenes*-positive sample in an active food distribution center *(8)*. The objective of this study was to determine if ATP bioluminescence levels were associated with *Listeria* spp., as these data were collected concurrently from environmental surfaces.

MATERIALS AND METHODS

Microbiological sampling

Seventeen distribution centers handling fresh produce were visited once between December 2019 and March 2021. These distribution centers represented two firms selected from a convenience sample of firms identified with assistance from the funding organization *(31)*. These centers handle items other than produce, such as meat, poultry, and nonconsumables. Approximately 18 environmental surface samples were collected per facility ($n = 300$) by using Sponge-Sticks with 10 ml of Dey-Engley neutralizing broth (3M, St. Paul, MN). Sample sites were chosen on the basis of the facility design, size, and general location (e.g., shipping and receiving docks, cold storage) to distribute samples collected throughout the facility. Only non-food-contact surfaces were sampled, such as floors, pallets, shelving, and equipment, using 30 by 30 cm areas. Foods and food contact surfaces (e.g., packaging) were not sampled as requested by the participating firms. However, non-food-contact surfaces serve as harborage sites, which can lead to cross contamination on food-contact surfaces. Samples were coded to blind the laboratory to collection site locations prior to microbiological analysis. After sampling, sponges

TABLE 1. Number of samples negative or positive for *Listeria* spp. along with the percentage of *Listeria* spp.-positive samples from the total number of samples collected per distribution center; mean log(x + 1) transformed RLU is also given per distribution center

Distribution center	DC1	DC2	DC3	DC4	DC5	DC6	DC7	DC8	DC9	DC10	DC11	DC12	DC13	DC14	DC15	DC16	DC17
Positive	2	0	2	0	0	2	0	2	3	0	0	0	0	0	1	0	1
Negative	18	16	17	19	21	17	11	9	12	20	16	14	27	21	19	14	16
Total	20	16	19	19	21	19	11	11	15	20	16	14	27	21	20	14	17
Percentage positive	10.0	0	10.5	0	0	10.5	0	18.2	20.0	0	0	0	0	0	5.0	0	5.9
Average log RLU	5.99	5.15	5.96	5.84	5.29	5.39	5.33	5.30	5.37	4.49	3.39	4.42	4.65	3.56	4.29	5.28	5.81

were returned to the original bag, sealed, stored on ice packs, and shipped to the laboratory for microbiological analysis. Upon receipt, insulated coolers (Uline, Pleasant Prairie, WI) were checked with an infrared thermometer (Fluke, Everett, WA) to confirm proper temperature was maintained during shipping, and samples were processed within 48 h of collection.

Each environmental sample was tested for *Listeria* species by using a modified version of the FDA *Bacteriological Analytical Manual* methodology for detection and isolation of *Listeria* in environmental samples *(15)*. Briefly, 90 ml of buffered *Listeria* enrichment broth (Difco, BD, Sparks, MD) was added to each bag with the sample, followed by homogenization in a Stomacher 400 Circulator Lab Blender (Seward, Worthing, UK) at 230 rpm for 60 s. A 360-µl aliquot of *Listeria* selective enrichment supplement was added to each sample bag after 4 h of incubation at 30°C. After incubation at 30°C for 24 and 48 h, a 50-µl aliquot of sample enrichment from each bag was streaked to BBL CHROMagar *Listeria* (Difco, BD) and Oxford medium base with modified Oxford antimicrobic supplement (Difco, BD) agars and incubated at 30 and 35°C, respectively, for 48 h. Up to four presumptive *Listeria*-positive colonies (blue-green colonies surrounded by an opaque, white halo for CHROMagar and brown-colored colonies with black zones for Oxford medium base with modified Oxford antimicrobic supplement) were selected for PCR confirmation by using *sigB* as the target gene *(31)*.

SuperSnap ATP swabs (Hygiena, Camarillo, CA) were also collected adjacent to environmental sample locations. For instance, if an area on a mop bucket was swabbed by using a sponge, a directly adjacent area of the bucket was used for ATP swabbing. Per the manufacturer's instructions, swabs were used to sample an area (10 by 10 cm). Areas were sampled by rotating the swab while sweeping across the surface vertically, horizontally, and then vertically. The swab was immediately placed in the Hygiena SystemSure Plus ATP Measurement System (Hygiena) testing chamber for measurement, and the displayed RLU level reading was recorded. In total, 300 ATP swabs were collected, with approximately 18 ATP swabs collected per facility.

Statistical analysis

RStudio (version 4.0.0) was used for statistical analyses by using the package readxl *(33)*. RLU data were log(x + 1) transformed to normalize right-skewed data. The analysis of variance function was used to determine the relationship between the predictor variable (log-transformed ATP) and outcome (*Listeria* spp. presence). Logistic regression analysis was performed by using the general linearized model function to determine if log(x + 1) transformed RLU levels were a predictor of *Listeria* spp. presence. Figures were created by using ggplot2 *(32)* and popbio *(26)*.

RESULTS AND DISCUSSION

This study was conducted by using a subset of distribution centers examined for prevalence of *Listeria* spp. *(29, 31)*. *Listeria* spp. were found in 12 of 17 distribution centers, with a total prevalence of ca. 13 (4.3%) of 300 environmental samples positive for *Listeria*. The prevalence of *Listeria* spp. in samples collected per distribution center ranged from 0 to 20% *(Table 1)*. Although six distribution centers did not have any *Listeria* spp.-positive samples, it is still possible that *Listeria* spp. were present in those facilities, as sampling did not occur on all surfaces. Accordingly, there may be differentiating factors influencing the likelihood or presence of *Listeria* within the distribution center environment. These factors may include facility characteristics, such as employee population, facility size, and amount of product handled annually. For instance, a larger employee population may lead to increased *Listeria* presence from shoe soles contaminated with naturally occurring *Listeria*. Several studies have assessed *Listeria* contamination on shoes *(17, 22)* and found that environmental soil is typically the main reservoir leading to contaminated shoe soles. In addition, factors, such as season, geography, and precipitation, were shown to be

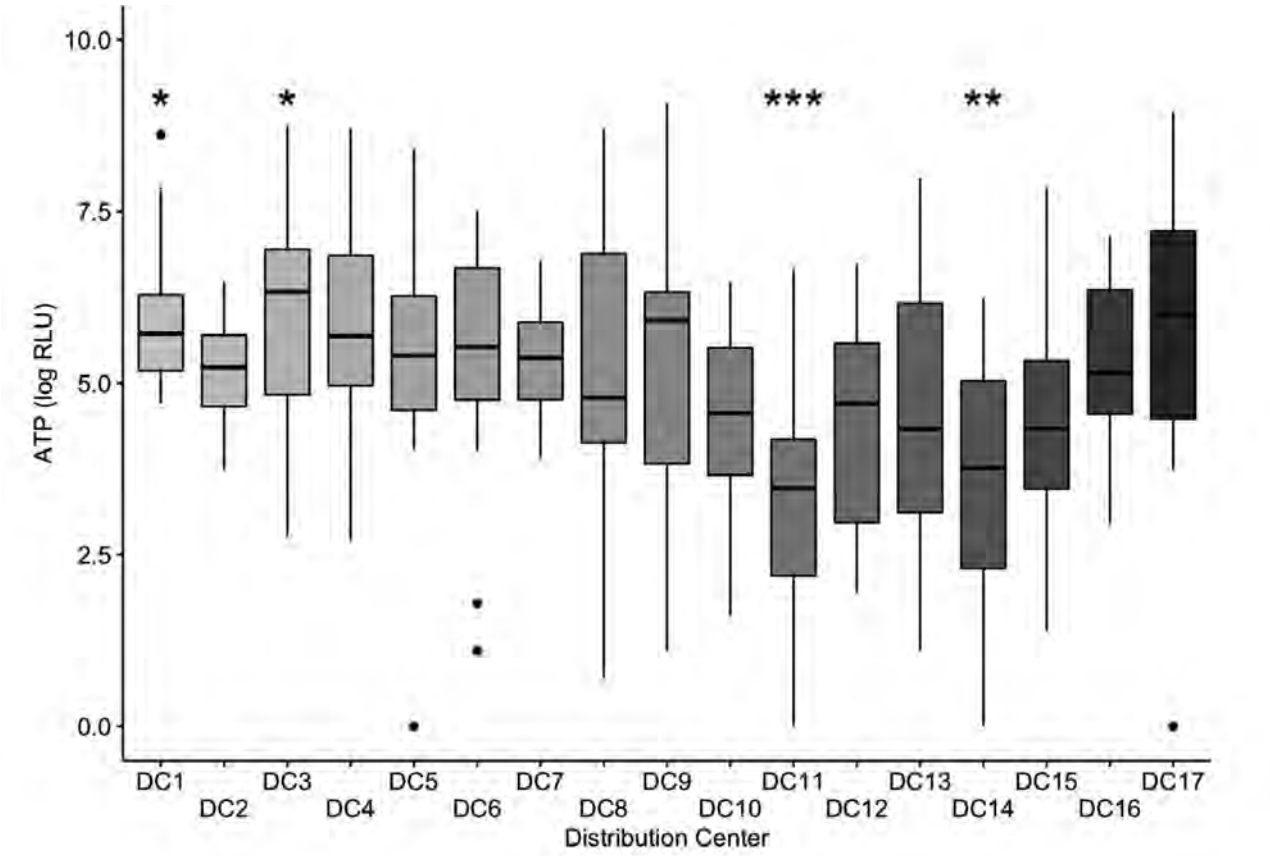

Figure 1. Box plots of log(x + 1) transformed ATP RLU across distribution centers. Significant differences between mean log(x + 1) transformed RLU across all distribution centers are noted by asterisks (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

significantly associated with *Listeria* presence in food-related environments *(8, 12, 27, 29, 31)*.

Untransformed ATP levels yielded a range of ca. 0 to 8,690 RLU, with a median and mean of ca. 166 and 559 RLU, respectively ($n = 300$). Log($x + 1$) transformed ATP levels ranged between 0 and 9.07, with a median of 5.12 and a mean of 5.01. Significant differences were observed between mean log RLU levels *(Fig. 1)* when comparing all distribution centers' mean log RLU in distribution center 1 (DC1) and DC3 ($P < 0.05$), DC11 ($P < 0.001$), and DC14 ($P < 0.01$; *Fig. 1*). DC1 and DC3 exhibited significantly greater mean log RLU levels (5.99 and 5.96, respectively), while DC11 and DC14 had significantly lower mean log RLU levels (3.39 and 3.56, respectively) across all distribution centers' mean log RLU levels.

The large variability of ATP RLU across sampling site locations within distribution centers is unsurprising. Other studies focused on microbial predictability or cleaning efficacy have observed variability in ATP levels across food and health care environments *(19, 28)*. ATP degradation may occur on the basis of other substances or factors, such as heat, enzymes, and acidic or alkaline compounds (e.g., cleaners, sanitizers), which may be present in the environment *(3)*. Therefore, ATP data may not always translate to available biomass or possible bacterial presence because of residual ATP degradation *(3)*. In addition, ATP stability on surfaces were investigated, where it was shown that surface-dried cultures of *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Candida albicans* retained 65 to 95% of their original ATP after 29 days *(2)*. However, this study used high inoculum concentrations (approximately 10^6 to 10^8 CFU per 0.1 mL/2 cm^2 surface), which may not necessarily be representative of the viable microbial population or load in food distribution center environments *(2)*.

When comparing average RLU levels and *Listeria* spp. prevalence within each distribution center, there does not appear to be an association between these two metrics. For example, greater mean RLU levels were not observed when the prevalence of *Listeria* spp. was high in a distribution center. A logistic regression *(Fig. 2)* for predicting a *Listeria* spp.-positive sample on the basis of log($x + 1$) transformed RLU levels provided an odds ratio of 0.34. Therefore, for each additional increase in 1 log RLU, the odds of a *Listeria*-positive sample increased by 0.34. The small magnitude of this odds ratio indicates that ATP is a poor predictor of a sample being positive for *Listeria* spp. in the survey of

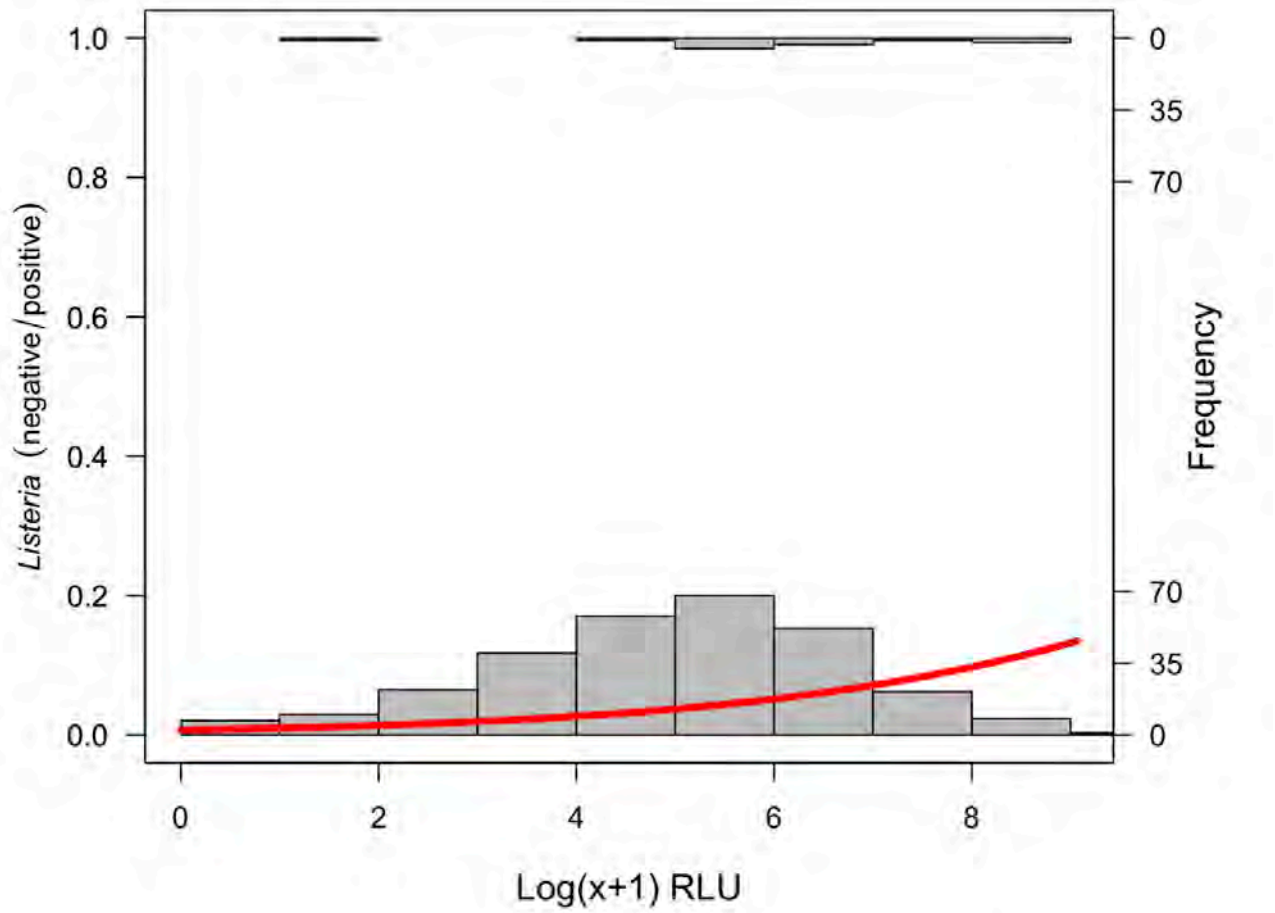

Figure 2. Logistic regression of log(x + 1) transformed ATP (predictor) data against *Listeria* presence (negative, 0; positive, 1) in samples with corresponding ATP bioluminescence data (n = 300).

distribution centers presented here. This odds ratio is low compared with other results from a study in retail delis *(14)*. Hammons et al. *(14)* determined that for every 1-log increase in preoperational mean ATP levels, the odds of detecting *L. monocytogenes* increased fourfold.

Several studies have compared various brands of commercial luminometers *(6, 16, 23)*. However, it is challenging to compare RLU levels for sensitivity, as RLU standardization can vary across luminometer brands. One study *(6)* compared four luminometers for applications in the brewing industry. None of the luminometers were reliable at detecting bacteria and yeast, with no consistent linear relationships observed between the number of CFUs and RLU, as well as fluctuating RLU levels between replicate samples *(6)*. Jago et al. *(16)* compared the performance of 10 commercial luminometers and found an 800-fold difference in ATP detection limit between the most and least sensitive electronic instruments. Furthermore, a comparison of three luminometers for health care-related surfaces observed ATP swab recovery of microorganisms varied by instrument and organism *(23)*. Therefore, individual differences within and between brands of ATP luminometers can contribute to variability in RLU measurements. As a result of this variability, food distribution centers and other food-related environments should conduct in-house validations to determine RLU thresholds for cleaning and sanitation protocols.

Although ATP cleanliness RLU thresholds (RLU levels) used in food industry applications may vary not only on the basis of the brand of luminometer and swabs *(3)*, but also ATP cleanliness thresholds may vary by the sample site surface material *(10, 25)*. For instance, on hospital-related surfaces, stainless steel showed the lowest ATP RLU levels, while melamine-coated surfaces and wood surfaces had the highest ATP RLU levels *(25)*. When ATP RLU threshold levels were used at university foodservice establishments, laminated surfaces had the highest rate of failure (97.2%), followed by stainless steel (88.5%), plastic (66.7%), and wood (57.1%) surfaces *(10)*. There may also be variation of RLU levels, not only by surface type, but also on the basis of

where those surfaces are used or located, such as in hospitals or food-related environments. For instance, laminate surfaces may have the highest failure rate on the basis of ATP RLU levels, compared with other surfaces, as laminate is commonly used to make cutting boards, and cutting boards are in direct contact with foods *(10)*.

CONCLUSIONS

Although ATP is often used to monitor and verify the cleanliness of surfaces and/or surface sanitation protocols, the data reported here suggest that ATP bioluminescent data were not reliable or appropriate predictors for *Listeria* spp. presence in sampled distribution centers. Because *Listeria* spp. were isolated from these distribution centers, *L. monocytogenes* may be present and pose a food safety hazard, if not properly detected and mitigated. Therefore, environmental monitoring programs should include strategies for microbial detection (or enumeration, if appropriate, on the basis of a microbial target) and not rely solely on ATP data to assess the overall sanitary condition in food distribution centers. ATP may have utility as part of a suite of tools that the industry is using but should not be used in isolation as an indicator of *Listeria* spp. in distribution center settings.

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