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Detection of Biological Contaminants on Foods and Food Surfaces Using Laser-Induced Breakdown Spectroscopy (LIBS)

Rosalie A. Multari,*[,†] David A. Cremers,[†] Jo Anne M. Dupre,[‡] and John E. Gustafson[§]

[†]Applied Research Associates, Incorporated, 4300 San Mateo Boulevard Northeast, Suite A-220, Albuquerque, New Mexico 87110, United States

[‡]Microbiology Group, Department of Biology, Molecular Biology Program, New Mexico State University, Las Cruces, New Mexico 88003, United States

[§]Department of Biochemistry and Molecular Biology, Oklahoma State University, 246 Noble Research Center, Stillwater, Oklahoma 74078-3035, United States

ABSTRACT: The rapid detection of biological contaminants, such as *Escherichia coli* O157:H7 and *Salmonella enterica*, on foods and food-processing surfaces is important to ensure food safety and streamline the food-monitoring process. Laser-induced breakdown spectroscopy (LIBS) is an ideal candidate technology for this application because sample preparation is minimal and results are available rapidly (seconds to minutes). Here, multivariate regression analysis of LIBS data is used to differentiate the live bacterial pathogens *E. coli* O157:H7 and *S. enterica* on various foods (eggshell, milk, bologna, ground beef, chicken, and lettuce) and surfaces (metal drain strainer and cutting board). The type (*E. coli* or *S. enterica*) of bacteria could be differentiated in all cases studied along with the metabolic state (viable or heat killed). This study provides data showing the potential of LIBS for the rapid identification of biological contaminants using spectra collected directly from foods and surfaces.

KEYWORDS: *E. coli, S. enterica, LIBS, multivariate regression, foods, surfaces, swab*

INTRODUCTION

The need to detect biological contaminants in foods and on food surfaces and the importance of this capability for food safety is well-understood and documented. Effective measures taken to ensure food safety often hinge on the ability to control the food processing environment and rapidly detect the presence of biological contaminants in foods and on food-processing surfaces.[1] *Escherichia coli* O157:H7 and *Salmonella enterica* are biological contaminants that have contributed to recent outbreaks of illness resulting from consuming contaminated foods. *E. coli* is a bacterium species commonly found in warm-blooded animals, and *E. coli* O157:H7 is a serotype that is known to cause illness in people. An *E. coli* O157:H7 infection can result in moderate to severe illness or death, with most of the deaths occurring in children under 5 years of age or the elderly,[2] and a common source of infection is beef; however, other foods, such as lettuce and raw milk, have also been shown to cause illness.[3] *S. enterica* is also responsible for severe illness and death in people. Common sources of infection are meat, poultry, eggs, milk, and products of eggs and milk.[4] The ability to rapidly detect the presence of these two biological contaminants is an important step in enhancing food safety.

Laser-induced breakdown spectroscopy (LIBS) is an analysis technique originally demonstrated in the early 1960s and is an outgrowth of atomic emission spectroscopy (ca. 1860), in which elemental composition was determined by placing samples in a flame and observing the resulting colors.[5] In LIBS, a laser plasma is used in place of a flame. The plasma is created by focusing laser pulses onto the surface of a sample using a lens. The plasma vaporizes and excites sample material (nanogram to microgram amounts), and the de-excitation/recombination light is observed (typically using a fiber optic to collect the light and direct it into a spectrometer). The collected light produces a spectrum that includes spectral signals from bulk (substrate) material, the sample surface, and the atmosphere surrounding the sample. Because all that is required to create the plasma is focused light, often no sample preparation is required for LIBS analysis. Over the years, LIBS has been applied to a broad range of applications by analyzing the spectrum for individual elemental emissions.[6−9] In recent

Table 1. Estimate of the Number of Cells Deposited in Each Contaminated Area for the Samples Used in This Study[a]

dilution	CFU in 100 μL
E. coli d5	3.7×10^4
E. coli d6	3.7×10^3
E. coli d7	3.7×10^2
E. coli d8	3.7×10^1
E. coli d9	3.7×10^0
S. enterica d4	9.7×10^5
S. enterica d5	9.7×10^4
S. enterica d6	9.7×10^3
S. enterica d7	9.7×10^2
S. enterica d8	9.7×10^1

[a]Control samples (uncontaminated) labeled as TSB d6, for example, contained the same amount of TSB used in a corresponding contaminated sample (e.g., *E. coli* d6).

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Table 2. Aerobic Plate Count Results for the Heat-Killed Pathogen Samples and the Uninoculated Food Samples[a]

dilution	CFU in 100 μL
heat-killed *E. coli*	0
heat-killed *S. enterica*	0
ground beef	TNTC
bologna	1.0×10^3
chicken	TNTC
milk	TNTC
eggshell	1.5×10^3
lettuce	2.5×10^3

[a]TNTC = too numerous to count.

The goal of this study is to expand our previous work in the use of LIBS with differentiation algorithms based on chemometric analysis for pesticide-related food safety applications to the detection of biological contaminants on foods and food-processing surfaces using the same methods of analysis. The specific objectives of this work are to demonstrate that mathematical analysis can be used to create prediction models to differentiate spectra or groups of spectra collected from samples to show that (1) contaminated samples can be differentiated from uncontaminated samples, (2) the type of contaminants can be identified, and (3) the metabolic state of the contaminant can be determined.

Table 3. Summary of All Samples and Dilutions Included in This Study[a]

sample	*E. coli*	*S. enterica*	HK[b] *E. coli*	HK[b] *S. enterica*	blank	TSB
ground beef	d5−d9		d5−d9		water	
bologna	d5−d9		d5−d9		water	d5−d9
chicken		d4−d8		d4−d8	water	
milk		d4−d8			milk	
eggshell	d5−d9	d4−d8	d5−d9	d4−d8	water	d5−d9
lettuce	d5−d9				water	d5−d9
drain	d5	d4	d5	d4	water	d4−d5
cutting board	d5	d4	d5	d4	water	d5
swab from eggshell		d4−d8			water	
swab from cutting board	d5	d4				

[a]See Table 1 for the number of cells applied to the samples at each dilution. [b]HK = heat killed.

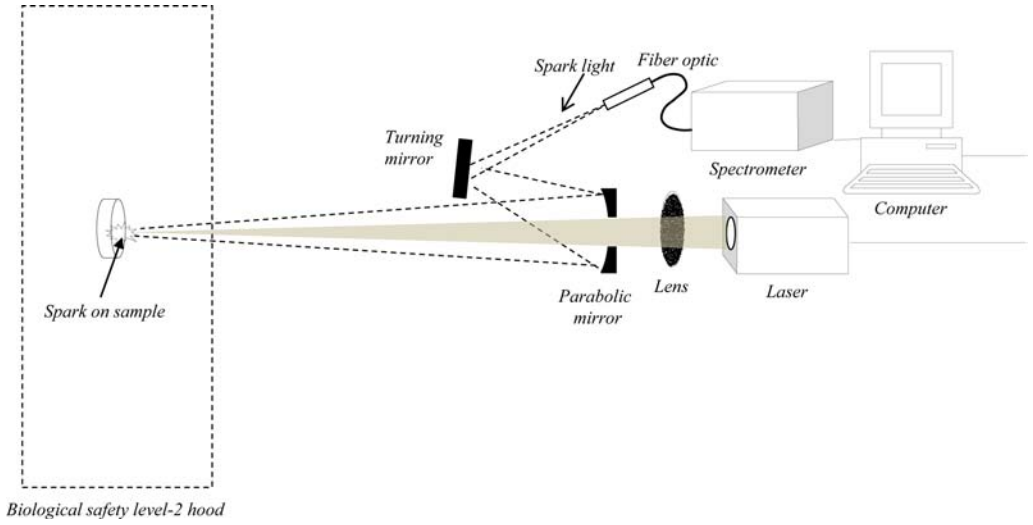

Figure 1. Experimental setup used to collect LIBS spectra. The samples were located inside a biosafety hood. LIBS emission was collected along the path of the laser light to remove parallax.

years, both classification and identification of material type have been accomplished by applying advanced chemometric techniques to the LIBS spectrum.[10] Numerous papers have described the use of chemometrics for the analysis of LIBS spectra for a variety of biological applications.[11−25] For food safety, the use of LIBS in combination with multivariate regression analysis has previously been applied successfully to the detection of pesticide contaminants in pellets made of powdered spinach and rice[26] and in the very complex and "dirty" (unknown and complex sample composition) matrices of tissue fats and rendering oils.[27]

MATERIALS AND METHODS

Food and surface samples were prepared with various concentrations of *E. coli* ATCC 4389[28,29] and *S. enterica* ATCC 8324,[30,31] as described below. Bacterial cultures were initiated from an isolated colony transferred from a trypticase soy agar (TSA, BBL, Sparks, MD) plate to 5 mL of tryptic soy broth (TSB) and incubated at 37 °C and 200 rpm, overnight. Broth growth was diluted in autoclaved deionized (DI) water for aerobic plate count enumeration and inoculation onto food surfaces ("contaminated"). For metabolic state investigations, duplicate 5 mL TSB cultures of *E. coli* and *S. enterica* were placed in a 95 °C water bath for 30 min. These heat-treated cultures, designated as "killed", were then diluted in DI water for inoculation onto foods

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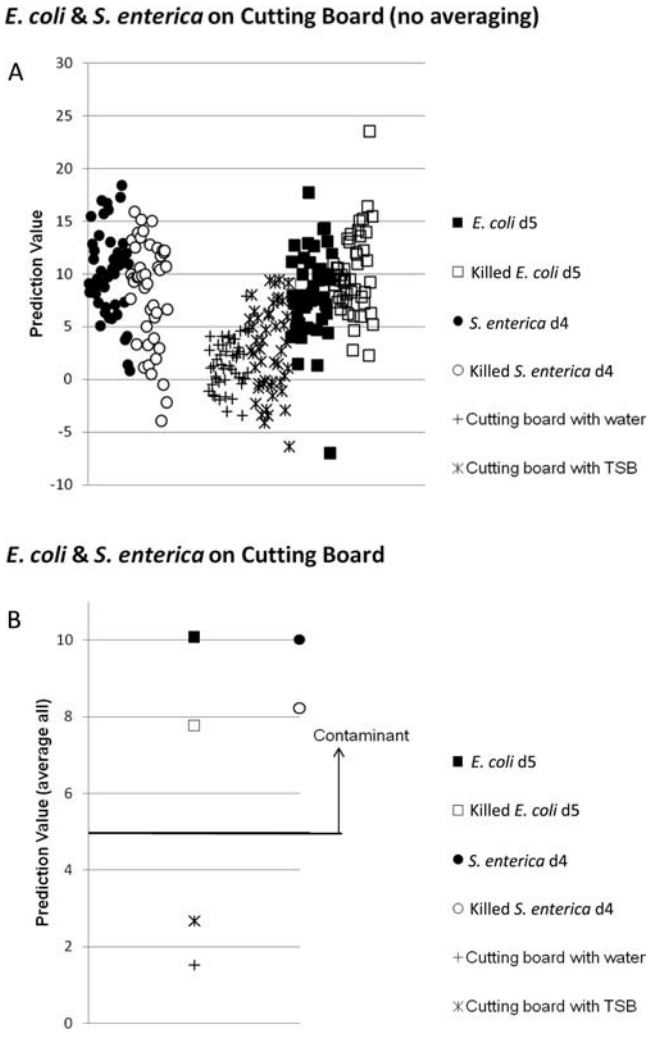

Figure 2. Prediction results obtained for inputting test spectra into a model designed to differentiate the contaminants *E. coli* and *S. enterica* independent of the metabolic state on a plastic cutting board from an uncontaminated cutting board (only water or TSB). Unaveraged prediction values are plotted in panel A, and averaged prediction values are plotted in panel B. For ease of viewing, some symbols have been displaced horizontally from each other.

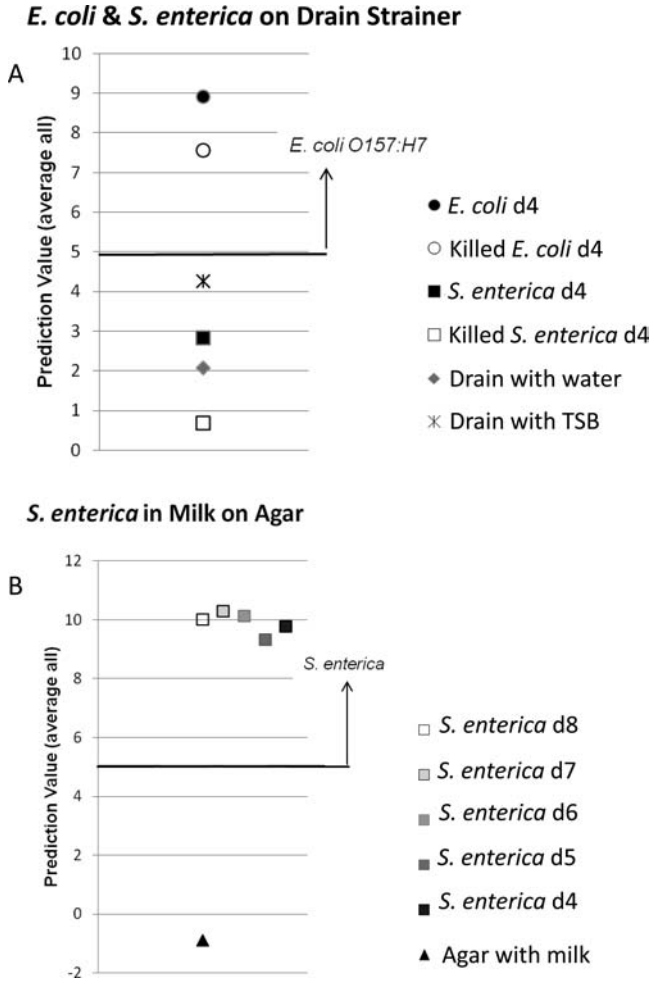

Figure 3. Prediction results obtained for inputting test spectra into a (A) model designed to differentiate *E. coli* independent of the metabolic state on a metal drain strainer (single dilution) and (B) model designed to differentiate *S. enterica* in milk across (a range of dilutions) from uncontaminated milk. For ease of viewing, some symbols have been displaced horizontally from each other.

and surfaces. Samples inoculated with either live or killed bacteria were considered "contaminated". As a control sample ("uncontaminated"), TSB was diluted in DI water and pipetted onto raw foods and surfaces. Samples inoculated with plain DI water were also included as "blank" controls ("uncontaminated").

Great care was taken in the sample preparation to ensure that the only difference between the contaminated and uncontaminated samples was the presence or absence of the bacteria. The ground beef, lettuce, bologna, eggs, chicken, plastic cutting board, and metal drain strainer used here were all purchased from local retail shops. All foods were stored at 4 °C in a chilling incubator (Ectotherm, Solana Beach, CA) when not in use. Food samples were divided into 4 × 2 cm (8 cm^2) sections aseptically using autoclaved utensils and placed in 100 mm^2 × 15 mm high disposable Petri dishes (VWR, Aurora, CO). Ground beef was removed from store packaging and transferred directly to Petri dishes. Romaine lettuce leaves were separated by cutting ~3.8 cm from the bottom core; the outer leaves were discarded; and the middle leaves were placed in sterile plastic bags and rinsed 4 times with DI water. This was followed by two rinses with 70% ethanol, and then the lettuce was allowed to dry on a sterile surface before being cut and transferred to Petri dishes. Bologna slices were transferred to the surface of an opened sterile plastic bag, cut, and then transferred to Petri dishes. Eggs were rinsed under running DI for 1 min, sprayed with 70% ethanol, allowed to dry on a sterile plastic bag surface in a biological safety cabinet, and then cracked (using clean gloved hands and a sterile knife) into a disposable Petri dish. Liquid egg was removed, and the eggshell was placed in Petri dishes and sprayed again with 70% ethanol. The shell pieces were broken using sterile utensils into sizes just small enough to lie flat, transferred to Petri dishes, and arranged to cover a 4 × 2 cm area. Raw chicken breast tenderloins (with up to 15% natural chicken broth, salt, and carrageenan) were rinsed under running DI water and placed in Petri dishes to dry in a biological safety cabinet. Reduced fat milk (2%) was pipetted into centrifuge tubes for inoculation before transfer to TSA plates. Non-food surfaces of the aluminum sink drain strainer and a nonporous plastic cutting board were inoculated in a 4 × 2 cm marked area by pipetting 100 μL of culture dilutions in small droplets and allowed to air-dry in a biological safety cabinet.

Replicate samples were inoculated by pipetting 100 μL of diluted *E. coli* or *S. enterica* over the entire 4 × 2 cm sample area. Droplets were spread over the surface using the pipet tip, and the area of inoculation was marked, so that LIBS data collection occurred only over the inoculated area for the contaminated samples or an uninoculated sample area for the uncontaminated samples. Various dilutions of pathogen samples were prepared to assess detection performance over a range of contamination levels. A portion of the prepared dilutions were heat-killed to assess the detection performance for different metabolic states. An estimate of the

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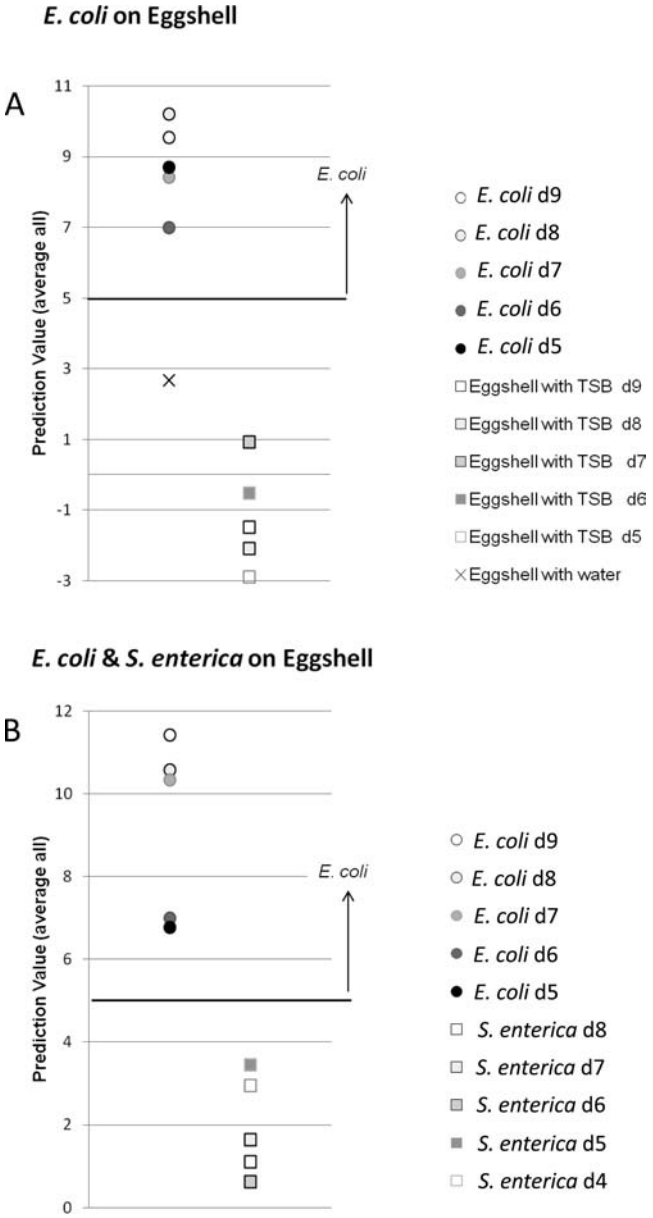

Figure 4. Prediction results across a range of dilutions obtained for inputting test spectra into a (A) model designed to differentiate *E. coli* on eggshell from control samples and (B) model designed to differentiate *E. coli* from *S. enterica* on eggshell. For ease of viewing, some symbols have been displaced horizontally from each other.

number of cells deposited in each contaminated area for the samples used in this study is found in Table 1. It should be noted that the TSB control samples, labeled, for example, as TSB d6, contained the same amount of TSB as an *E. coli* contaminated sample, labeled as *E. coli* d6.

Aerobic plate counts of each uninoculated food sample were used to detect any background microflora. For the plate counts, 1 g of food sample from each package was aseptically weighed into a 50 mL conical tube to which was added 9 mL of 0.1 M sodium phosphate buffer (pH 6.8). The tube was vigorously vortexed for 1 min, and then 1 mL was plated on TSB agar plates. The first dilution (d1) was diluted again 10-fold and plated. Plates were incubated at 37 °C and colony forming units (CFU) per milliliter were determined after a period of 24 h. In cases where no growth was detected, the plates were incubated for an additional 24 h and re-examined. The aerobic plate count results for the heat-killed pathogen samples and the uninoculated food samples are found in Table 2. As seen from this table, all uncontaminated food samples had unknown bacteria present on the samples prior to inoculation and the heat-killed dilutions had no viable cells.

For swab testing, sterile cotton-tipped swabs (Puritan Medical Products Company, LLC, Guilford, ME) were dipped into a 50 mL conical tube of autoclaved DI water and pressed against the side of the tube to remove excess water. A swab was then rubbed across the inoculated surface area of a sample. Swab tests were performed by wiping the surface area with the swab 4−5 times, then turning the swab, and repeating several times. A summary of all of the samples and dilutions used in this study is found in Table 3; however, only a subset of the predictive plots have been included here as needed to illustrate the differentiation capability of the analysis methods used in this study.

LIBS data were collected directly from the foods, surfaces, and swabs of the foods and surfaces using the experimental setup presented in Figure 1 previously described.[32,33] Pulses (1064 nm wavelength, 60 mJ/pulse, and 10 Hz repetition rate) from a model CFR 400 Q-switched Nd:YAG laser (Big Sky Laser, Bozeman, MT) were focused onto the sample, which was manually held in the laser beam path. Because this study involved viable bacterial contaminations, the samples were positioned inside a biological safety level-2 hood during the data collection. LIBS data were collected directly from the surface of the solid food surface and swab samples. The liquid milk sample was spread on agar plates to prevent splashing of contaminated material during data collection. Plasma light was collected using an off-axis parabolic mirror and a fiber optic and then routed to a model AvaSpec-2048-2-USB2 dual channel fiber-optic spectrometer (Avantes, Broomfield, CO). A hole in the parabolic mirror permitted the optical path of the laser pulses and light collection to be collinear, eliminating parallax. Each recorded spectrum represented the average of 10 spectra (detector acquisition parameters: 1 μs delay and 1.1 ms window). A total of ∼100 spectra were recorded from each sample.

Commercially available software, The Unscrambler (Camo Software, Inc., Woodbridge, NJ), was used for analysis, and detailed accounts of how analysis was accomplished have been published previously.[27,32−34] The method of analysis employed was partial least-squares regression with only one response variable (PLS1). This is a method of analysis in which the variations in one response variable (Y variable) are related to the variation of the predictors (X variables). The X-variable set used in the analysis was each wavelength channel of the spectrometer detector for the entire wavelength range measured (4096 variables resulting from the use of two spectrometer channels: channel 1, 232−494 nm and 0.32 nm resolution; channel 2, 495−1026 nm and 0.6 nm resolution). The Y variable associated with each channel was the intensity measured by the individual spectrometer channel.

RESULTS AND DISCUSSION

The LIBS spectrum involves combined signals arising from the sample (contaminant plus substrate) and the atmosphere surrounding the sample with these signals dependent upon the experimental conditions used to acquire the spectrum, such as laser pulse energy, lens to sample distance, etc. The analysis methods applied in this work are applicable only to the differentiation of a sample type from a predefined sample group. No claim is being made that absolute detection of the biological target has been accomplished here. What is being demonstrated is the ability to differentially identify a sample or group of samples from a predefined set using well-defined experimental conditions. Such analysis is useful for developing LIBS instruments for specific applications in which experimental conditions can be fixed and in which the samples to be differentiated can be characterized such that the natural sample variability is captured in the detection algorithm (i.e. the range of the variation in sample types is understood). That is, the differentiation models developed are applicable only to the equipment configuration used to collect these data and the specific foods, surfaces, and type of swab used. The detection algorithm development methodology for different

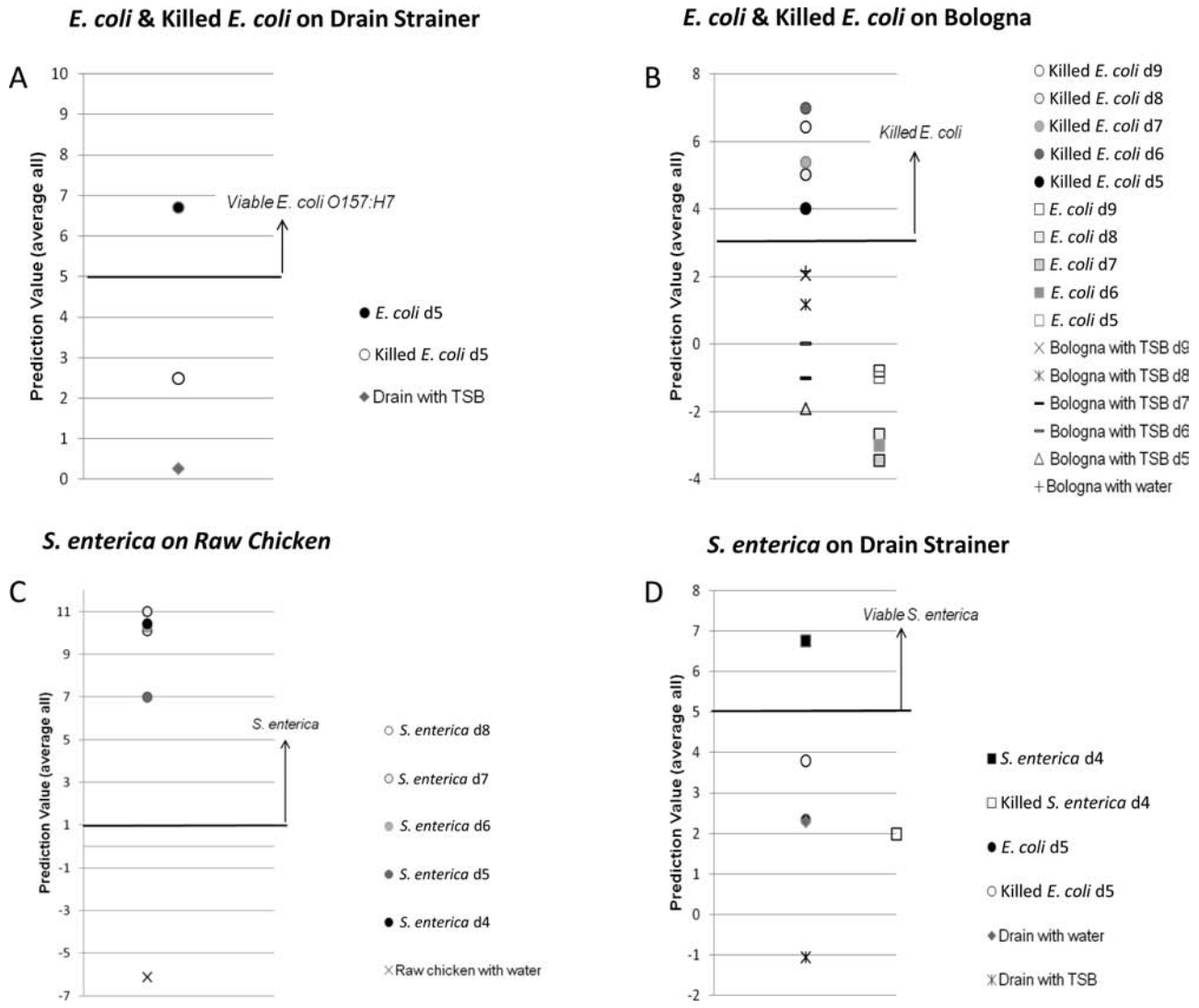

Figure 5. Prediction results obtained for inputting test spectra into a (A) model designed to differentiate viable *E. coli* on a metal drain strainer from killed *E. coli* and a control (single dilution), (B) killed *E. coli* on bologna from viable *E. coli* and controls (range of dilutions), (C) *S. enterica* on raw chicken (range of dilutions) from raw uncontaminated chicken, and (D) live *S. enterica* on drain strainer from killed *S. enterica*, killed *E. coli*, viable *E. coli*, and controls (single dilution). For ease of viewing, some symbols have been displaced horizontally from each other.

equipment configurations or different substrate foods and surfaces would be the same, however.

The differentiation models are based on single-variable partial least-squares regression combined with principal component analysis applied to the recorded LIBS spectrum. Through an iterative estimation technique, a general model is developed that includes canonical correlation, redundancy analysis, multiple regression, multivariate analysis of variance, and principal components. In this case, only one dependent variable is being modeled. Once a satisfactory model has been generated, it is used to produce a "prediction value" (in this case, between 0 and 10) that is used to match the sample being tested to one of the samples included in the modeling. The entire LIBS spectrum from each sample was analyzed to develop differentiation algorithms, and no attempt was made to correlate detection to specific atomic or molecular signatures from the targeted species. Attempts at reducing or limiting the spectral range being modeled produced poorer prediction results than those presented here.

Spectral normalization (the maximum peak value within each spectrum was equal to 1 following the normalization) was applied to all spectra prior to multivariate analysis. To ensure that the modeling did not overfit the data, cross-validation included in The Unscrambler software was used in the model build and the number of components chosen for the prediction was selected as suggested by the software. In the cross-validation, one sample at a time was kept out of the calibration and used for prediction. This process was repeated until all samples were kept out once. The validation residual variance was computed from the prediction residuals to assess error and determine the appropriate number of components to use in the prediction to avoid overfitting the data.

For each sample group differentiated, models were created using half of the total number of spectra collected (model build spectra). Typically ~100 spectra were collected per sample. The spectra not used for the model (~50) were reserved and subsequently used to test and evaluate model performance (verification spectra). The output of the model when a spectrum was input was a "prediction value" that was correlated to how well

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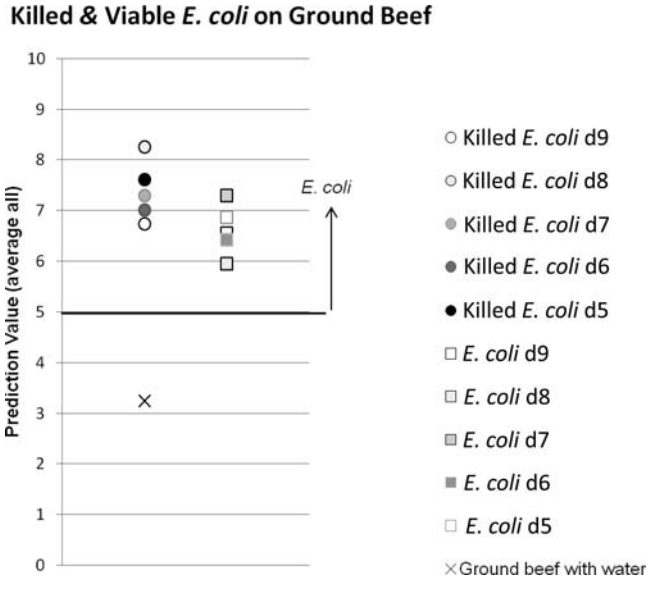

Figure 6. Prediction results obtained from inputting test spectra into a model designed to differentiate killed *E. coli* on ground beef from a control (range of dilutions). For ease of viewing, some symbols have been displaced horizontally from each other.

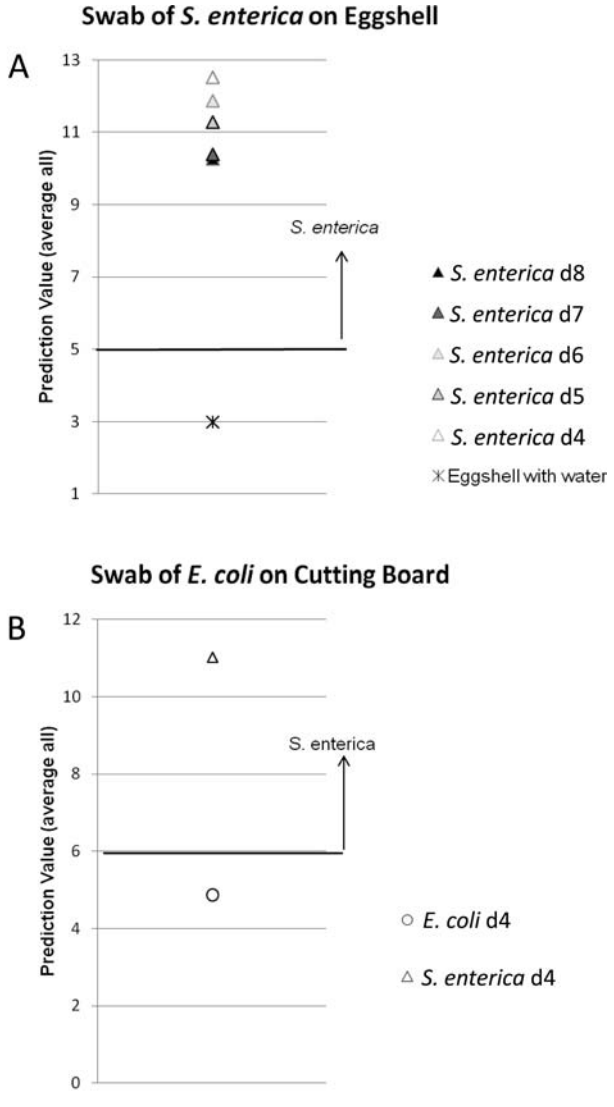

Figure 7. Prediction results obtained from inputting test spectra into a (A) model to differentiate a swab of *S. enterica* on eggshell (range of dilutions) from a control swab and (B) model to differentiate a swab of *E. coli* on a cutting board from a swab of *S. enterica* on a cutting board (single dilution).

the input sample matched the sample groups used to build the model. Good discrimination models were defined as those that resulted in prediction values for the samples being discriminated such that the sample could be unambiguously associated with the groups that the model was designed to differentiate. Samples with high prediction values were associated with the group being differentiated (e.g., *E. coli* on eggshell) from the other groups that were not differentiated among themselves (e.g., TSB on eggshell and eggshell with water), with these being characterized by low prediction values. Here, the models were developed so that the prediction value range spanned 0−10. The choice of the prediction value range used in the modeling, however, does not affect the prediction results because the impact of choosing a different range would just be different maximum and minimum values on the predictive plots with the prediction values distributed on the plots with the same relationship to each other over the range selected. To improve the observed separation in prediction values, the prediction values obtained by analyzing the verification spectra for a particular sample (∼50) were averaged (this is indicated by "average all" in the plots that follow) and the average prediction values were then used to identify the samples. Figure 2 illustrates the impact of averaging the prediction values prior to differentiating the sample groups. Unaveraged prediction values are plotted in Figure 2A, and averaged prediction values are plotted in Figure 2B. As seen, without averaging, there is no clear separation between the sample groups upon which to build a detection algorithm suitable for deployment on an instrument. After averaging, however, there is a separation between groups and a prediction value can be chosen to differentiate the samples.

For all samples, it was found possible to build chemometric models to differentiate contaminated from uncontaminated samples. Figure 2 shows prediction results obtained for inputting verification spectra (spectra not included in the model building) into a model designed to differentiate between *E. coli* and *S. enterica* independent of the metabolic state on a plastic cutting board from an uncontaminated cutting board (only water or TSB). This model was created by including spectra from both the viable and heat-killed *E. coli* and *S. enterica* samples (single dilution) in the modeling as the "care" set (high prediction value) and the control samples of water and TSB on the cutting board as the "don't care" set (low prediction value). As shown, a gap in the prediction values exists such that a line can be drawn above which all of the contaminated samples fall.

The ability to differentiate the type of contamination was also investigated, and it was found possible to differentiate the contamination type for all of the samples and sample dilutions studied. Figure 3 shows prediction values obtained for a model designed to differentiate *E. coli* independent of the metabolic state on an aluminum metal drain strainer (viable and killed *E. coli* were used as the "care" set, with controls and *S. enterica* as the "don't care" set in the modeling) and for a model designed to differentiate the entire concentration range of *S. enterica* in milk ("care" set) from uncontaminated milk ("don't care" set). It is also interesting to note that, in plot A, there is a gap between the viable *E. coli* and killed *E. coli* prediction values. On an instrument control system, an algorithm could be implemented using this model that uses one prediction value to

first identify the type of contamination followed by a second prediction value analysis to subsequently identify the metabolic state of the detected *E. coli*.

Figure 4 shows prediction results for a model designed to differentiate a range of *E. coli* dilutions on eggshell from control samples and a model designed to differentiate *E. coli* from *S. enterica* on eggshell (dilutions were used as the "care" set in each case). As shown, the differentiation models can be designed to differentiate single dilutions or across a range of dilutions as desired. It should be noted that, because the differentiation is based on a prediction value calculated using modeling designed to produce a matching value based on spectral similarities and differences to the groups being modeled, it is possible to produce prediction values above and below the range used in the modeling. When prediction values outside the range are produced (e.g., plot A), this indicates that the input spectra are dissimilar in some respect to the spectra used to develop the model. This is not an issue for using the predictive model in an instrument control system as long as the unknown samples are binning high or low correctly.

An investigation was also made into the ability to differentiate the metabolic state, and it was found possible to differentiate either killed or viable *E. coli*. Figure 5A shows prediction value results for models designed to differentiate viable *E. coli* on a metal drain strainer from killed *E. coli*, and Figure 5B shows differentiation of killed *E. coli* on bologna from viable *E. coli* and controls (range of dilutions). The metabolic state and type of contamination could also be differentiated simultaneously if desired. Figure 5C shows prediction value results for a model designed to differentiate *S. enterica* on raw chicken (range of dilutions) from raw uncontaminated chicken, and Figure 5D shows results from a model to differentiate live *S. enterica* on a drain strainer from killed *S. enterica*, killed *E. coli*, viable *E. coli*, and controls (single dilution). In addition, it was found that differentiation models could be created using only killed dilutions that also differentiated viable dilutions even though the viable dilutions were not included in the modeling. Figure 6 shows prediction results obtained from inputting test spectra into a model designed to differentiate killed *E. coli* on ground beef (range of dilutions) from a control. Note viable *E. coli* is differentiated even though it was not included in the model building. Similar results were obtained for differentiating *E. coli* on lettuce from uncontaminated lettuce.

Lastly, the ability to differentiate samples using LIBS data collected by sparking on swabs that were used to wipe selected surfaces was investigated. Differentiation was demonstrated for all cases studied. Figure 7A shows prediction results obtained from inputting test spectra into a model designed to differentiate a swab used to sample *S. enterica* on eggshell (range of dilutions) from a control swab. Figure 7B shows results obtained for a model designed to differentiate a swab used to sample *E. coli* on a plastic cutting board from a swab that sampled *S. enterica* on a plastic cutting board (single dilution). As shown, the differentiation results obtained using swab sampling produced results similar to those obtained using LIBS data collected directly from the samples.

This study extends previous work dealing with identification of pesticides and dioxins in tissue fat and rendered oils[27] by employing the same methods to differentiate biological contaminations on foods and food-processing surfaces. It is demonstrated that LIBS also has potential as a rapid diagnostic for the detection of biological contaminants. This approach is different from previously published works in that the detection developed here does not determine the presence of the bacteria through the use of specific emissions believed to be characteristic only of the bacteria. Instead, the methodology uses a signature characteristic of the bacteria on the food or surface when sampled with a certain instrument configuration and specific instrument settings. More work is needed to fully explore the robustness of analysis using the LIBS spectra that are combinations of the bacteria, matrix, and surrounding atmosphere that are then analyzed using an algorithm developed specifically for the application, where the algorithm is a combination of discrete discrimination models. The unit cost of a LIBS instrument is predicted to be comparable to upper end PCR instruments. The main advantages of LIBS-based technology for this application will be the speed of analysis, minimal sample preparation, use of few consumables, and ability to detect pathogens on all types of surfaces. On the basis of our previous work[27,32,33] and the work described here, we predict that, once the algorithm has been developed and loaded into a LIBS instrument, a sample could be analyzed in about 3 min.

AUTHOR INFORMATION

Corresponding Author

*Telephone: 505-816-6312. Fax: 505-872-0794. E-mail: rmultari@ara.com.

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Notes

The authors declare no competing financial interest.

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