

Estimating Extent of Bacterial Injury by Impedance Measurement

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ABSTRACT

A method is described to estimate bacterial injury based on impedance measurements using modern automated systems. The system avoids the labor intensive systems that are based on estimations of colony forming units (CFU) on both non-inhibitory and inhibitory media. Estimates can be made using standard curves for non-injured cells to predict from observed detection times the expected versus the actual observed CFU numbers. The difference between these values represents the injured population.

Numerous methods have been used to estimate the extent of recoverable versus non-recoverable or lethal injury to bacterial cells resulting from various treatments. Traditional plate counts on non-inhibitory complete media typically have been employed to estimate the total populations of bacteria surviving the treatment, and by adding osmotic stress agents such as elevated concentrations of sodium chloride to the non-inhibitory medium an estimate of the proportion of the total population that is injured is obtained. These techniques are relative in that addition of yeast extract, catalase, pyruvate, and other agents to the "non-inhibitory medium" may markedly improve or increase the number of colony forming units (CFU) found on the plates. Mackey and Derrick (1982) described the effect of sublethal injury resulting from heating, freezing, drying, and gamma-radiation on the duration of the lag phase of *Salmonella typhimurium*. They used standard plate counts to obtain data to which growth curves were fitted using the Gompertz function. Such methods are extremely labor intensive.

Mackey and Derrick (1984) discovered that the lag phase of injured was greater than that of uninjured *S. typhimurium* by conductance measurements. The

purpose of this presentation manuscript is to describe the application of the observations of Mackey and Derrick (1984) to obtain estimates of radiation and heat injury using modern impedance instrumentation. For this purpose a limited study of heat injury of *S. typhi-murium* ATCC 14028 in ground turkey meat was completed that included, for purposes of comparison, estimation of injury by plate counts on trypticase soy agar containing 3% NaCl. In addition, the results of studies of protection provided to *S. typhimurium* by the presence of NaCl during treatment with gamma radiation and the injury of *Bacillus cereus* by gamma radiation will be described.

METHODS

S. typhimurium ATCC 14028 was obtained from the American Type Culture Collection, Rockville, MD, and maintained on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) and cultivated aerobically in tryptic soy broth (TSB)(Difco) at 37°C. Culture identity and purity of this serovar were verified by Gram stain and biochemical reactions using the Vitek Automicrobic System® GNI card (bioMérieux Vitek, Inc., Hazelwood, MO)(Knight et al. 1990), and confirmed by serologic testing with polyvalent and individual O-group antisera (Fisher Diagnostics Salmonella Diagnostic Sera, Fisher Scientific Co., Orangeburg, NY).

One milliliter from a 15 to 18 h culture of *S. typhimurium* ATCC 14028 in TSB was used to inoculate 100 ml of fresh TSB in a 500 ml baffled shake flask. The culture was incubated with shaking (150 rpm) at 37°C for 16 h and harvested by centrifugation and finally resuspended in 1/10 volume of Butterfield's phosphate.

Ten milliliters of the cell suspension were used to inoculate 100 g of radiation sterilized (42 kGy, in

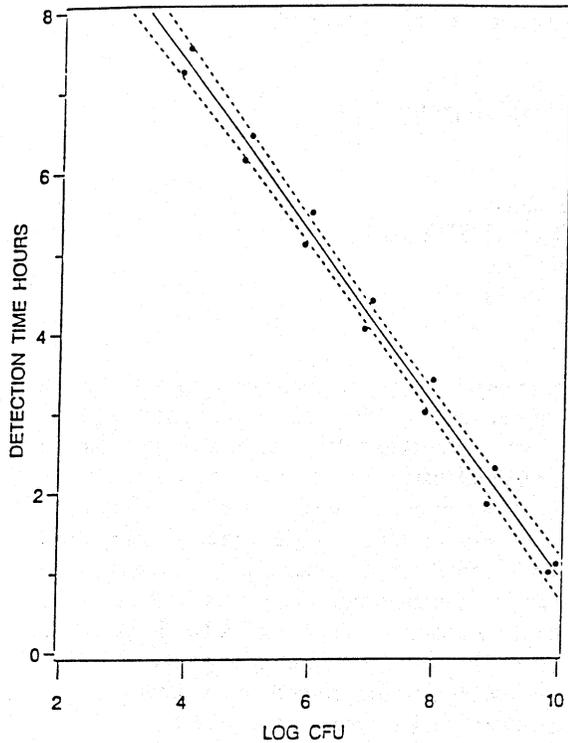


Figure 1. Standard curve for detection of uninjured *S. typhimurium* by impedance measurement at 37°C. The dashed lines represent the 95% confidence limits for the regression.

vacuo, at -30°C) ground turkey meat. The inoculum was mixed well with the meat by stomaching for 90 sec. The inoculated meat was then distributed aseptically in 5.0 ± 0.05 g amounts into sterile polyethylene Stomacher 400 bags and spread thinly over an area of approximately 10 x 10 cm; finally each bag was vacuum sealed. Heat treatments were applied to the samples within the bags by rapidly submersing the entire bag into a well mixed water bath maintained at 60 ± 0.05 °C. This was accomplished with a wire frame that held the bag flat as well as submerged in the water. When the appropriate amount of time had passed the sample bag was removed from the water bath and quickly submerged in an ice water bath. The time required for the samples to reach 60°C was approximately 15 sec.

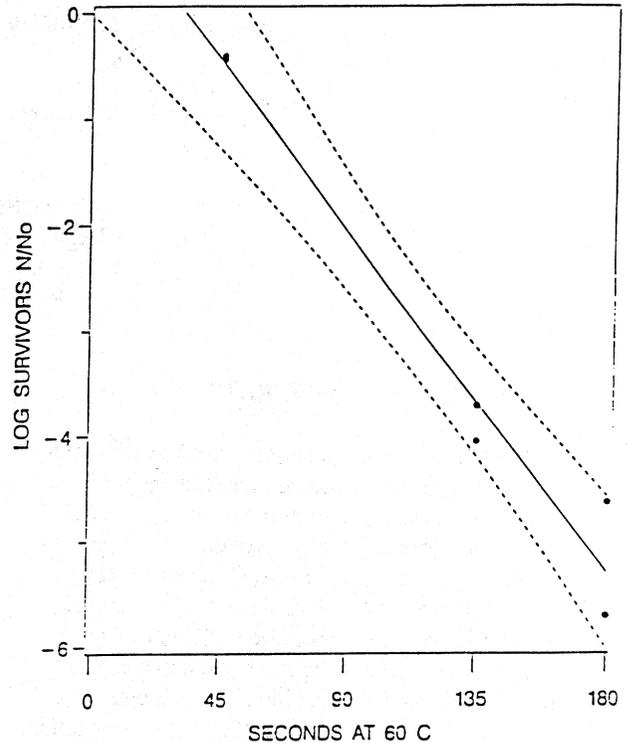


Figure 2. Effect of heating *S. typhimurium* at 60°C in ground turkey meat on survival. The dashed lines represent the 95% confidence limits for the regression.

A one to ten dilution of the sample was prepared by directly adding 45 ml of sterile Butterfield's phosphate buffer to each sample and stomaching for 90 sec. Subsequent dilutions were also prepared in the same diluent. Estimates of total viable CFU were made by standard pour plate techniques using TSA with incubation for 48 h at 37°C before counting using a Biotran II automated colony counter (New Brunswick Scientific, Edison, NJ). Estimates of the populations of injured cells were made by Spiral Plate technology using TSA containing 3% NaCl as the stress agent. These counts were made after 24 h incubation.

Impedance analyses were performed using the bioMérieux Vitek Bactometer. Samples (1.0 ml) for assay of detection time were withdrawn from the 10^{-2} dilution that had been used to prepare the plate

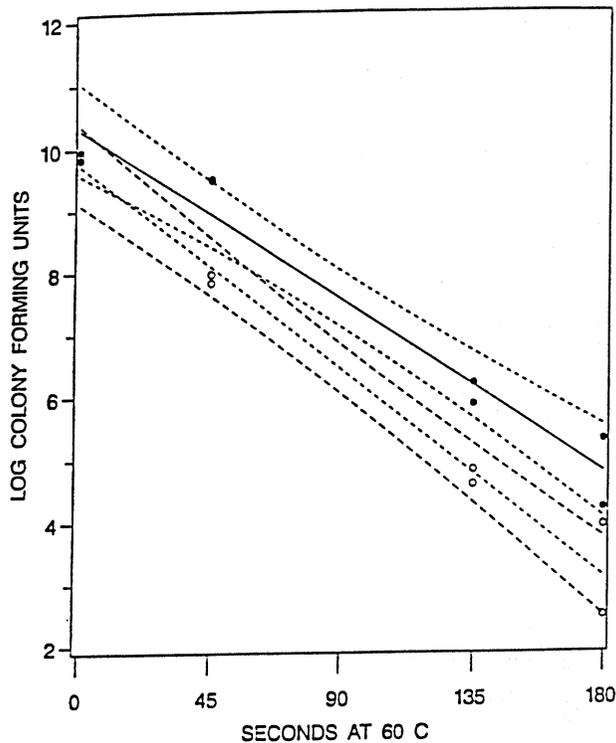


Figure 3. Observed (●) versus predicted (○) logarithm CFU for heated *S. typhimurium*. Predicted value based on detection time. The dashed lines represent the 95% confidence limits for the regressions.

counts and mixed with 9 ml of Wilkins-Chalgren Anaerobe Broth (Oxoid, Ltd., Basingstoke, Hampshire, England). Two milliliter samples were placed into two assay wells of the Bactometer plate to determine impedance detection time at 37°C. Detection times for both uninjured and injured cells were determined for a wide range of populations by using serial dilutions of samples from each study in Wilkins-Chalgren broth. In each case the population estimate was based on the results of the pour plate assay. A standard curve was prepared for the uninjured cells by plotting CFU against detection time (Fig. 1). Similar standard curves for detection time versus CFU were prepared for the heated cells. Population estimates can be made from each detection time measured by reference to

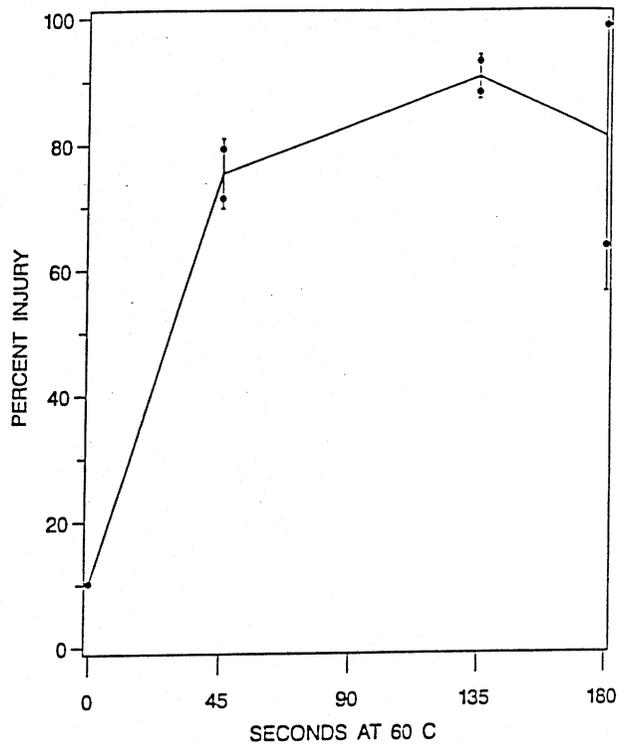


Figure 4. Estimated percent injury of *S. typhimurium* caused by heating at 60°C. Estimate based on prediction from detection time versus observed CFU.

the standard curve for detection time versus CFU for uninjured cells. In a similar manner estimates of expected detection times can be made from actual CFU measurements. The differences between the actual CFUs, estimated by plate counts on TSA, and CFU values predicted from measured detection times represent the injured population. The assumption is that injured cells require more time to initiate log-phase growth than do uninjured cells even in the extremely good repair Wilkins-Chalgren medium which contains pyruvic acid. Because detection time depends on both the initial number of cells per unit volume and the lag time, we do not equate the detection time with lag time (Mackey and Derrick, 1984). Lag times can however be estimated from detection times (Mackey and Derrick, 1984).

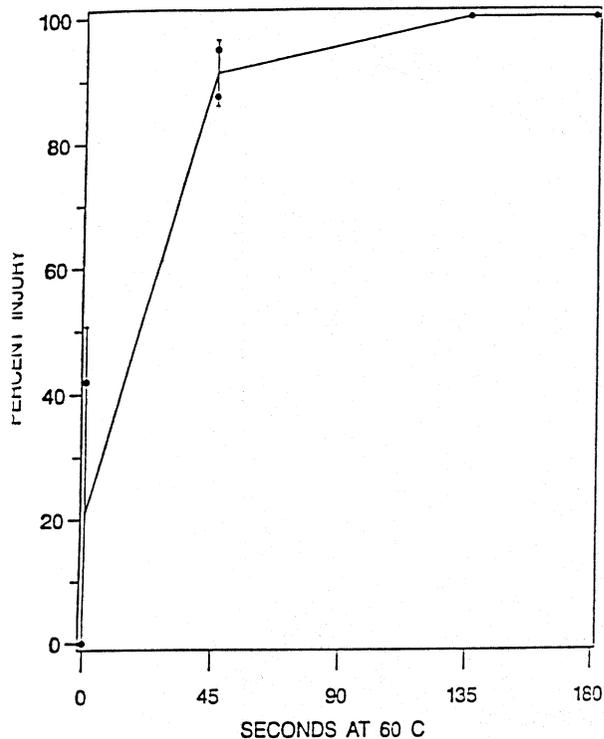


Figure 5. Estimate of percent injury to *S. typhimurium* resulting from heating at 60°C measured by relative growth of uninjured cells on TSA containing 3% NaCl.

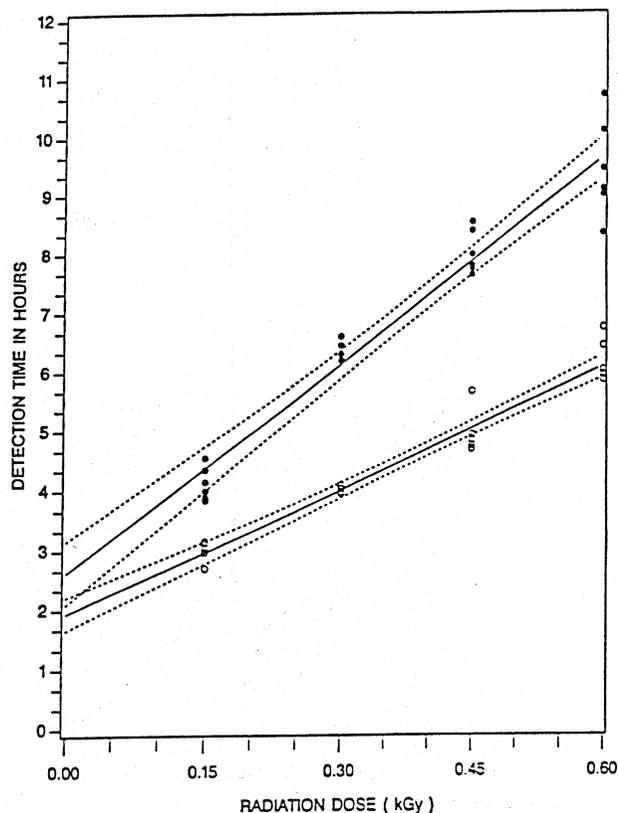


Figure 6. Impedance detection times (●) and predicted detection times (○) for irradiated *B. cereus* ATCC 33018 logarithmic-phase cells. The dashed lines represent the 95% confidence limits for the regressions. Adopted from Thayer and Boyd (1994).

Statistical analysis is an essential component in the interpretation of the results. We chose to use the linear regression (REG) procedure of the Statistical Analysis System (SAS) statistical package (Freund et al., 1986; SAS Institute, 1987) for our analyses. Estimates of populations were expressed as the logarithm of the number of CFU per g of turkey. A number of analyses must be made in sequence to interpret the results.

RESULTS AND DISCUSSION

The initial calculation is the determination of the survival curve for heated *S. typhimurium*. The survival value (N/N_0) that is the number of CFU (N) divided by the initial number of CFU (N_0). The negative reciprocal of the slope of the regression of

the logarithm N/N_0 plotted against heating time (Fig. 2) is the D_{10} -value (28.1 ± 2.5 sec).

The regression analysis of the logarithm of CFU against time, in sec, produces an equation that predicts the logarithm of CFU surviving each heat treatment. The regression equation for the standard curve (Fig. 1) for non-heated cells versus detection time is used to predict values for the logarithm of the CFU based on observed detection times. The results are shown graphically in Fig. 3. The difference between the observed and predicted values represents the injured population. Since these values are logarithms of the CFU they must be converted to the antilog values to calculate percent injury (Fig 4). The results are similar to estimates based on CFUs on TSA+3% NaCl (Fig 5).

Estimations of slopes for the regressions of detection times in hours versus logarithm CFU were -1.054, -1.205, -1.434, and -1.602 for cells heated for 0, 45, 135, and 180 sec, respectively. The slope for detection time versus CFU therefor provide a separate indication of the relative injury sustained by the cells.

From such measurements of impedance detection time Thayer et al. (1995) found that there was an inverse correlation between the molality of suspending NaCl solutions and the amount of injury sustained by *S. typhimurium* ATCC 14028 irradiated to 0.8 kGy at 5.0°C. Injury was estimated to be 26, 28, 27, 32, 32, 41, and 49% at 4.03, 2.83, 1.77, 1.20, 0.607, 0.150, and 0 molality, respectively. Thayer and Boyd (1994) used impedance detection times to estimate the amount of injury to log-phase *Bacillus cereus* cells subjected to relatively small doses of gamma radiation (Fig. 6).

The authors conclude that impedance techniques can provide a relatively simple and rapid method for estimating bacterial injury, whereby a relatively large number of samples can be examined during an experiment without the labor associated with conventional plate counts using inhibitory media.

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