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STATISTICAL VALIDATION OF THE TRACK-DILUTION PLATING METHOD FROM GROUND BEEF AND CARCASS SURFACE SAMPLES[1]

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ABSTRACT

A rapid and easy method of obtaining a viable culture count, the Track-Dilution method, was evaluated and compared to Spiral Plating to obtain viable culture counts from ground beef samples and beef carcass surface tissue samples inoculated with salmonellae or Escherichia coli *O157:H7. Based on the statistical analysis of the dataset (n = 125 samples), the Track-Dilution method results were not different (P > 0.1) than those obtained from Spiral Plating. Linear correlation of the scatterplot of Spiral Plating and Track-Dilution indicated a high level of agreement between these two methods (r^2 = 0.97). The Track-Dilution method was a valid method for estimating viable culture counts for meat animal-derived samples with bacterial counts of more than 100 cfu/mL.*

INTRODUCTION

Microbiology laboratories rely on the viable culture count as a technique for estimating bacterial populations. Several different methods and variations for conducting bacterial plate counts are used (Swanson *et al.* 1992; Koch 1994). Jett *et al.* (1997) published a method, termed the Track-Dilution method, for performing plate counts on a routine basis. This technique was compared to spread plating and was found to be highly economical in terms of time and resources. The

[1]Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Track-Dilution method uses a basic volume of 10 μL spotted to an agar surface (square plates recommended) which is then tilted to allow the droplet to move downward by gravity and distribute its contents over the lane of the plate. The Track-Dilution method has a theoretical lower limit of detection of 100 colony forming units (cfu) per mL when performed in a single determination.

The purpose of this study was to test the Track-Dilution method for enumerating bacteria from inoculated food animal samples and to collect a number of data points on which to perform a statistical analysis to validate this method for future use as a research tool.

MATERIALS AND METHODS

Organisms

Escherichia coli O157:H7 and *Salmonella choleraesuis* subsp. *choleraesuis* Serotype *typhimurium* (ATCC 14028), were maintained as part of the culture collection of the Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE, as glycerol suspensions at −20C. Cultures were prepared by subculturing in LB broth or tryptic soy broth + 0.5% w/v yeast extract at 37C for 16-24 h. All media components were obtained from Difco Labs (Detroit, MI) or BBL (Cockeysville, MD).

Sample Sources

Samples for analysis were obtained from a variety of sources. Lean and adipose beef carcass tissues were collected from carcass flank sections and stored frozen until thawed for use. Ground beef (90%) lean was stored frozen until thawed and tempered for use. In several on going experiments, samples that were normally tested by only Spiral Plating were split and analyzed by the Track-Dilution method as well in order to have side-by-side comparisons.

Preparation of Agar Plates

For the Track-Dilution method sterile, tempered agar (described in Table 1) was poured into square Petri plates (100 × 100 mm; Lab-Tek/NUNC; NUNC, Inc., Naperville, IL) and allowed to solidify. Cooled, solidified plates were dried for at least 15 min under a laminar flow hood, with plate lids opened. LB agar (Difco Laboratories, Detroit, MI) and Rambach agar (Gene-Trak Systems, Framingham, MA, distributors) were supplemented with ampicillin (100 μg/mL) obtained from Sigma Chemical Co. (St. Louis, MO).

TABLE 1.
SOURCE AND DESCRIPTION OF INOCULATED SAMPLES FOR COMPARISON OF THE TRACK-DILUTION METHOD AND SPIRAL PLATING

Menstrua	Inoculum	Treatment	Plating agar
Lean beef carcass surface tissue[a]	*E. coli* O157:H7	(none)	LB agar + 100 μg/ml amp[c]
Lean beef carcass surface tissue	*E. coli* O157:H7	Washed once in 10 ml of sterile water	LB agar + 100 μg/ml amp
Lean beef carcass surface tissue	*E. coli* O157:H7	Washed twice in 10 ml of sterile water	LB agar + 100 μg/ml amp
Adipose beef carcass surface tissue[a]	*E. coli* O157:H7	(none)	LB agar + 100 μg/ml amp
Adipose beef carcass surface tissue	*E. coli* O157:H7	Washed once in 10 ml of sterile water	LB agar + 100 μg/ml amp
Adipose beef carcass surface tissue	*E. coli* O157:H7	Washed twice in 10 ml of sterile water	LB agar + 100 μg/ml amp
Ground beef[b]	*Salmonella typhimurium*	(none)	Rambach agar

[a]After treatment, samples processed by stomaching for 2 min in a fiber filtered Stomacher bag with 25 mL of buffered peptone water + 0.05% Tween 20 as a Stomaching buffer. All tissue sections measured 2 × 6 cm)
[b]Samples processed by stomaching 25 g of ground beef in 225 mL of Stomaching buffer (see footnote[a]) for 2 min in a fiber filtered Stomacher bag.
[c]amp = Ampicillin

Plates for Spiral Plating were prepared in standard 100 × 15 mm round Petri dishes. Spiral plating was accomplished as previously described (Siragusa *et al.* 1995) using a Model D Spiral Plater (Spiral Biotech, Inc., Bethesda, MD).

Track-Dilution Method

Samples to be plated were serially tenfold diluted in buffered peptone water (Difco, Detroit, MI). A 10-μL sample from each dilution was deposited on an imaginary line on one end of a square prepoured plate of each of the respective agars. After depositing the last sample, the plate was tilted approximately 80° by placing the plate into a multi-pipettor sample reservoir (as a holder) with edge

opposite the inoculated edge downward. The droplets would then gravitate downward until all of the sample was thinly deposited over a straight line. Plates were allowed to stay in the multi-pipettor sample reservoir for at least 1 - 2 min, or until all of the other plates in the series were inoculated. When using properly dried plates, at no time in this study did any of the droplets spread on the opposite edge of the plate. All plates were incubated for 24-36 h aerobically at 37C before counting. Lanes with the highest number of readily distinguishable and separate colonies were used to obtain the final count. All samples were plated in duplicate.

Statistical Analysis

All bacterial counts were normalized to cfu/mL and converted to the equivalent $\log_{10}$ expressed to two significant digits. Individual values of duplicate data were compared to assess reproducibility. The mean of duplicates was used for comparing the Track-Dilution and Spiral Plating methods. Linear regression and Student's t-tests were performed using the statistical package of Microsoft Excel 97 (Microsoft, Redmond, WA) and InStat2 version 2.0 statistical analysis package (GraphPad Software, San Diego, CA).

RESULTS AND DISCUSSION

A total of 125 samples from various sources (Table 1) was plated by the Track-Dilution method and Spiral Plating.

A comparison of the two plating methods by linear regression analysis of the scatterplot of inoculated sample data (Fig. 1) indicated a high degree of correlation ($r^2 = 0.97$, $P < 0.001$) between Spiral Plating and Track-Dilution. The paired t-test statistical analysis (Table 2) indicated no significant difference ($P = 0.635$) between Spiral Plating and Track-Dilution. Analysis of the individual counts composing the duplicate data indicates that the level of agreement between duplicate determinations was as high for Track-Dilution ($r^2 = 0.98$) as that calculated for Spiral Plating ($r^2 = 0.96$).

The greatest disadvantage to the Track-Dilution method is its relative lack of sensitivity. Sensitivity of viable bacterial plating methods is dependent on the sample volume. As presented in this study and the original report (Jett *et al.* 1997), the lower limit of sensitivity of the Track-Dilution method is 100 cfu/mL from a 10 μL sample, as compared to 50 cfu/mL from a 50 μL sample of Spiral Plating. Plating techniques such as pour plating, membrane filtration and surface spread plating have even lower thresholds of detection, all of which are sample volume-dependent (Swanson *et al.* 1992; Koch 1994). The drop plate method is similar to Track-Dilution in that several drops from a calibrated pipette are deposited on an agar surface and allowed to dry before incubation. This method, which is reported

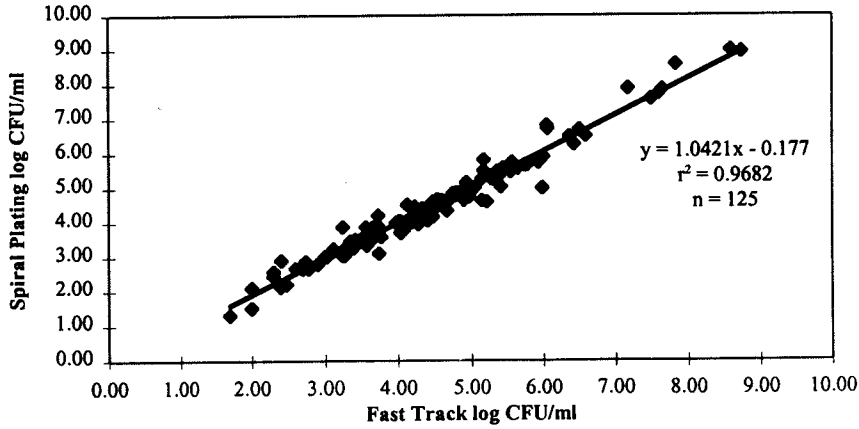

FIG. 1. SCATTERPLOT WITH LINEAR TREND LINE FOR TRACK-DILUTION PLATING AND SPIRAL PLATING

TABLE 2.
PAIRED T-TEST OF THE MEANS OF LOG CFU/ML OBTAINED FROM SPIRAL PLATING AND TRACK-DILUTION PLATING METHODS OF MEAT ANIMAL SAMPLES

Parameter	Track-Dilution	Spiral Plating	Value
Mean	4.45	4.46	--
Number of points	125	125	--
S.E.M.	0.122	0.129	--
Mean of paired differences	--	--	-0.0113
Student's t	--	--	0.4822
Degrees of freedom	--	--	124
Two tailed *P* value	--	--	0.6305

to have a threshold of at least 3,000 cfu/g, does not allow for the deposition of cells along a track of agar surface to facilitate easier counting (Swanson *et al.* 1992). All of the aforementioned can be made more sensitive by increasing replicative determinations of the same samples and, in effect, increasing the total volume plated. In this respect the Track-Dilution method shows its greatest advantage in that performing up to six replicate determinations of the same sample dilution requires only a single plate. Plating greater than 10 μL resulted in sample tracks that accumulated at the bottom end of the square plate and, therefore, was unacceptable.

A method previously reported (Fung and LaGrange 1969) utilized a microtiter plate dilution step followed by deposition of 25 μL to 50 μL of a diluted sample on a standard petri dish of agar medium without subsequent spreading of the inoculum. These workers reported a high level of agreement between their method and a standard plate count method for manufacturing-grade and grade-A milk.

Considerations of time, expenditure, cost and overall logistic advantages of the Track-Dilution method and the microtiter method were previously discussed (Fung and LaGrange 1969; Jett *et al.* 1997). In brief, these methods require lower medium volumes and fewer plates, since six dilutions of the same sample or six different samples may be applied to the same plate (Jett *et al.* 1997). Perhaps the greatest advantage offered by the Track-Dilution method is the per-sample plating time; plating six samples requires only about 1 min.

Considering the question of sensitivity, its utility should primarily be as a research tool to estimate bacterial concentrations in samples which have a level of bacterial cells known to be more than 100 cfu/mL. As previously reported (Jett *et al.* 1997), the reproducibility and accuracy, when compared to spread plating, were equal for a variety of bacterial species in pure cultures including *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. This method provides an extremely efficient and time-saving means to perform large numbers of viable bacterial counts. The current set of data indicates that Track-Dilution plating has a precision and accuracy and reproducibility similar to another widely accepted method, Spiral Plating, when used for sponge samples from animal carcasses and ground beef samples.

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