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Visual Demerit and Microbiological Evaluation of Beef Carcasses: Methodology

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ABSTRACT

A repeatable, automated method was developed for estimating aerobic bacterial populations on surfaces of groups of beef carcasses. Ten sample cluster sites (CS) were identified by localizing visual demerits (Canadian Streamlined Inspection System) on 200 carcasses at one plant. Most probable number growth units per cm^2 (MPNGU/cm^2) on hydrophobic grid membrane filters (HGMF) were assessed by an automated HGMF interpreter for excision samples from the centers of these CS. Between-sample variation of more than 90% of the total $\log_{10}$ MPNGU/cm^2 variance indicated good repeatability between HGMF of the same sample and interpretations of the same HGMF. Variance component estimates indicated that there was considerable variation in MPNGU/cm^2 between carcasses and between paired adjacent samples for a CS. A statistically significant but weak association was found between the demerit scores of a CS and MPNGU at its center. The variance component estimates will be used to estimate the sample size required for future group-carcass evaluations.

The food industry uses technology which permits the humane slaughter of cattle and dressing of carcasses at single line speeds as high as 450 carcasses per h. Agriculture Canada is working with industry to develop quality control and food safety enhancement programs in order to permit this modern advancement and to ensure the safety of the final product.

Thus, the Canadian Streamlined Inspection System for cattle (CANSIS-C), modified from that of the United States, was introduced on a trial basis in 1989 (*2*). CANSIS-C is designed for heifer and steer slaughter inspection and operates only in those high-speed plants that have initiated an approved partial quality control program. It relies predominantly on organoleptic methods for identifying pathological changes and aesthetic demerits. CANSIS-C applies finished product standards to all edible products at the last critical control point before leaving the kill-floor. These standards include the counting and measuring of carcass demerits at the end of the dressing line but not the level of bacterial surface contamination.

The objectives of the study were fourfold:

a) To establish and verify the repeatability of a microbiological protocol using hydrophobic grid membrane filters (HGMF) technology.

b) To examine the variability in aerobic colony counts expressed as most probable number of growth units (MPNGU): i) between adjacent samples at selected sites, ii) between sites on the same carcasses, and iii) between carcasses.

c) To summarize the distribution of CANSIS-C visual demerits on randomly selected carcasses.

d) To assess the agreement between the visual demerit scores and MPNGU at sites.

The methodology described in detail in this study will be used to estimate levels of bacterial contamination on groups of carcasses at the last critical control point of the kill-floor. Using this technology, further studies are required to establish sample size and microbiological criteria for the verification of Hazard Analysis Critical Control Point (HACCP) systems. The HACCP system is internationally recognized as a systematic approach to be used in food production as a means to assure food safety and is espoused by Agriculture Canada in its Food Safety Enhancement Program (*16*).

MATERIAL AND METHODS

Enumeration of aerobic colonies

Samples used to determine the protocol for bacterial enumeration were taken from carcasses of two plants (n = 126). Sample source is not relevant to protocol development. Subsets of the samples were used to examine different microbiological protocols, described below, for estimating MPNGU. Carcass surface samples (5 x 5 x 0.5 cm) were excised using sterile forceps and scalpel blades. The corners of the area to be sampled were marked with

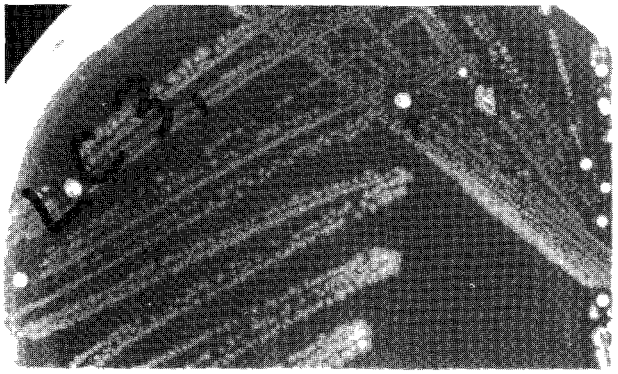

Figure 1. *Soldering iron with four brass rods used to mark the corners of samples to be excised from carcass surfaces.*

a branding iron (175 W soldering iron), at the end of which were four brass "L"-shaped rods (3 mm diameter) (Fig. 1). The four "L's" marked the corners of the samples (light burn marks remained on the carcass). The temperature at the end of the brass rods was 122°C. The forceps were sterilized by squeezing the shaft of the branding iron (260°C). Excised samples were placed in stomacher bags (10-15 cm, H 3681-50, Baxter Corporation), and the bags were suspended individually, 2 cm apart by "double clips" from a rack inside a cooler, 1/3 filled with crushed ice. The samples were 5 cm above the ice and at a temperature of 4 ± 1°C on arrival at the laboratory. Samples were processed in the laboratory 3-4 h after sampling.

Within 1 h of arrival at the laboratory, sample bags were removed from the cooler, 30 ml of 0.01% peptone (Difco) - 0.1% Tween 80 (Sigma Chemical Co.) was added, and the sample was washed for 10 or 30 s in a Stomacher (Colworth 80) at a 4.5-mm hammer setting (120 strikes per 10 s). A sample of the suspension was withdrawn by pipette with a pipette-tip prefilter of 110-μm mesh (Filtaflex, Almonte, Ontario). Two milliliters of the prefiltered suspension with particles <25 μm was spread evenly on one or more HGMF (ISO-Grid, QA Life Sciences, San Diego, CA) including their borders by means of the pipette after the prefilter was discarded. Vacuum suction was applied to the HGMF placed on a Spreadfilter (Richard Branker Research Ltd., 27 Monk St., Ottawa, Canada). After filtration (10-200 s, filtration time was reduced by pipette-tip prefilter which was without effect on MPNGU, n = 50, P > 0.10), the HGMF was transferred with sterile forceps onto the dry surface of tryptic soy agar (TS) (20 ml in plastic petri dishes - 15 x 100 mm) with 0.01, 0.005, or 0.0025% of 2, 3, 5 triphenyltetrazolium chloride (TTC, Sigma Chemical Co., T 8877, TTC was added to the media after autoclaving), or 0.025% Fast Green indicator (0.25 g Fast Green FCF/L, C.I #42053, Sigma Chemical Co. Cat. No. F 7252) in TS was tested similarly. Plates were incubated (unhumidified) at 35°C for 24 or 42 h. HGMF were interpreted automatically by a MI-100 Interpreter System (Richard Branker Research Ltd.) with individual adjustment of filters (red, green, blue, or none), camera focus, and aperture in order to achieve maximum "flagging" (interpretation) of colonies, as seen on the monitor. This would ensure the highest possible "Z" positive number (guide of reliability) for each MPNGU calculation. The reliability of automatic interpretation of the HGMF was verified by duplicate interpretation of HGMF (n = 32). HGMF preparation and the principles of automated interpretation have been described (*18*).

In order to evaluate the effect of TTC and Fast Green indicators on bacterial growth, strains of 24 different bacterial species[1] (17 genera) were streaked onto TST plates (TS with 0.005% TTC, see below) and TS plates with Fast Green (0.25%) and incubated at 37°C for 42 h. In addition, colonies from 16 carcass samples were grown on HGMF with or without TTC. Sixty of these colonies grown on eight HGMF incubated 42 h on TST plates (0.005% TTC) and 69 colonies grown on eight HGMF incubated 18 h on TS plates were selected at random for identification.

Analyses of variance (*19*) were carried out to determine the effect of stomacher time (10 versus 31 s), and incubation length (24 versus 42 h at 35°C), on the $\log_{10}$ MPNGU/cm^2 (LMPN) when TST plates (0.01% TTC, Difco Manual 1984 p. 939) were used. A split-plot statistical model was used with effects due to sample and stomacher time included in the main-plot analysis and effects due to incubation length and its interaction with stomacher time in the subplot analysis. A similar analysis was used to compare the TS plates with Fast Green (0.025%) versus TST plates at the two incubation times. For samples where MPNGU were determined for repeat HGMF or duplicate interpretations per HGMF, estimates of variance components (*19*) for samples, HGMF within samples, and interpretations within HGMF were determined for the LMPN. The VARCOMP procedure in the SAS (SAS Institute, Inc.) software (*17*) was used to carry out the calculations using the method of moments (*13*). The repeatability or intraclass correlation is the ratio of the between sample variance component to the total variance of an observation.

The LMPN for 30 carcass samples were compared for HGMF incubated for 42 h on plates with 0.01% or 0.005% TTC using analysis of variance and accounting for the effects due to sample and concentration. The LMPN for 30 other carcass samples were compared similarly for TST plates with 0.01% or 0.0025% TTC. The LMPN means for the concentrations were transformed back to the original MPNGU/cm^2 scale as geometric means.

Plant

One modern CANSIS inspected abattoir was available for the demerit localization and demerit versus MPNGU/cm^2 studies. A brief description of the plant is offered to be able to relate the results to the conditions of carcass dressing. The uniform type of beef cattle slaughtered at this plant is restricted to grain finished heifers or steers of less than 24 months of age. Lairage time is less than 12 h. The "hide-on" floor (22 x 44 x 30 m) is separate from the "hide-off" floor (32 x 65 x 30 m) except for an opening at one corner for the passage of the carcass line. The line speed was 180/h at which speed the time taken by a carcass to pass through the "hide-on" floor (68 carcasses) and the "hide-off" floor (42 carcasses) is approximately 30 min. Approximately 72 workers are on the line from the start of the "hide-on" floor to the end of the "hide-off" floor. There are about seven quality control staff and six Agriculture Canada inspectors on the floors at any one time. Hand-wash basins and implement sanitizers are available at all work stations. All carcasses were studied at the end of the dressing line before the carcass wash.

Localization of CANSIS demerits on carcass surfaces

CANSIS Finished Product Standards for Beef Carcasses are applied randomly at the end of the trimming line. They comprise 10 nonconformance classifications and are designed to assure that the slaughter and evisceration processes are in conformance with Agriculture Canada standards. Four of these are of possible

[1]*Aeromonas hydrophila, Agrobacterium tumrifaciens, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Enterobacter cloacae, Erysipelothrix rhuseopathiae, Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Micrococcus luteus, Pasteurella haemolytica, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas testosteroni, Rhodococcus equi, Salmonella montevideo, Salmonella typhimurium, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae, Streptococcus faecalis, Streptococcus pyogenes.*

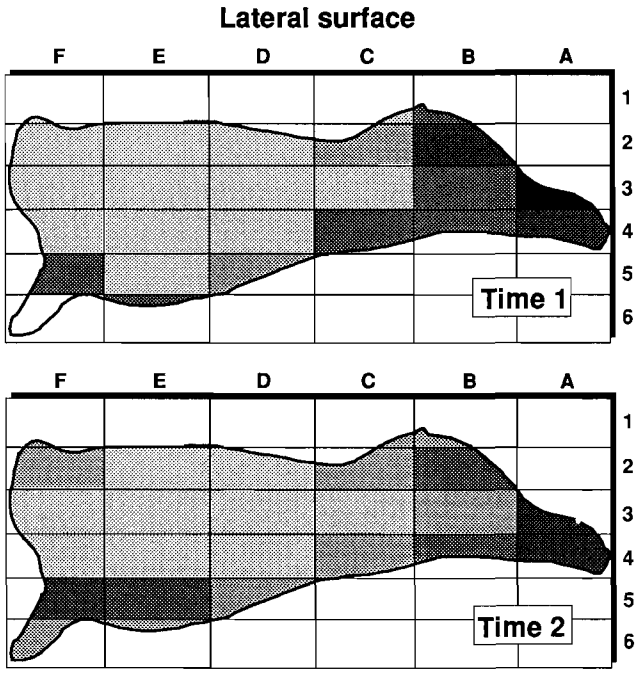

Figure 2. *Lateral aspect of beef carcass with 24 arbitrarily defined regions in which the locations of demerits were recorded for each carcass. Cluster analyses of demerit scores of regions from 100 carcasses from each of the two studies revealed six clusters. These clusters are shown by the densities of the shading, with dark shading representing high demerit scores. For example, for study (time 1) regions A3 and F2 had demerit scores of 106 and 7, respectively. Region F6 was not included in study (time) 1.*

relevance to bacterial contamination: specks, dressing nonconformance (smears and stains resulting from contact with ingesta, hide, personnel, other carcasses, etc.), hair strands, and hair clusters and these were used in this study (*2*). CANSIS-trained AC inspectors and plant quality assurance personnel were asked to plot the location of the four CANSIS demerits within arbitrarily sized regions on diagrams depicting the lateral (24 regions) and medial (15 regions) aspects of the carcasses (Fig. 2). A subjective code of 0-3 (none to numerous visual demerits) per region was used to translate the four relevant nonconformances into carcass regional scores. Only lateral carcass surface demerits were considered and therefore the medial abdominal and thoracic surfaces were excluded. The carcasses (randomly identified and railed out by plant staff) for this study were also objectively evaluated by CANSIS. To determine if the results would be consistentt over time, 100 carcasses were assessed in studies 1 and 2, four months apart. A composite diagram of demerits by region was created for each 100 carcasses (Fig, 2). Study 1 included 11 plant visits and subjective evaluation by 12 workers. Study 2 was done by one inspector in eight visits. For each study, an analysis of variance (*19*) was carried out on the demerits per region accounting for effects due to carcass and region. An analysis was also carried out over studies with effects due to study and the study x region interaction added to the statistical model. A cluster analysis using the CLUSTER procedure in SAS (*17*) was carried out on the mean demerit score for each region to obtain approximate groups of regions with similar scores.

Sites for microbiological sampling

The distribution of demerits on the composite diagram revealed the occurrence of demerits in clusters which crossed regional borders. Accordingly, 10 cluster sites (CS) of high and low total demerit density were delineated on the lateral surface of the carcass, each with the following dimensions in cm: hock (10 x 30), rump lateral (15 x 30), rump medial (15 x 30), rectum (15 x 25), flank (45 x width of flank), thorax (25 x width of thorax), brisket (10 x 40), axilla (6 x 40), neck (25 x 45), and shank (15 x 30). Two adjacent samples were taken for MPNGU determination at the center of each CS (Fig. 3).

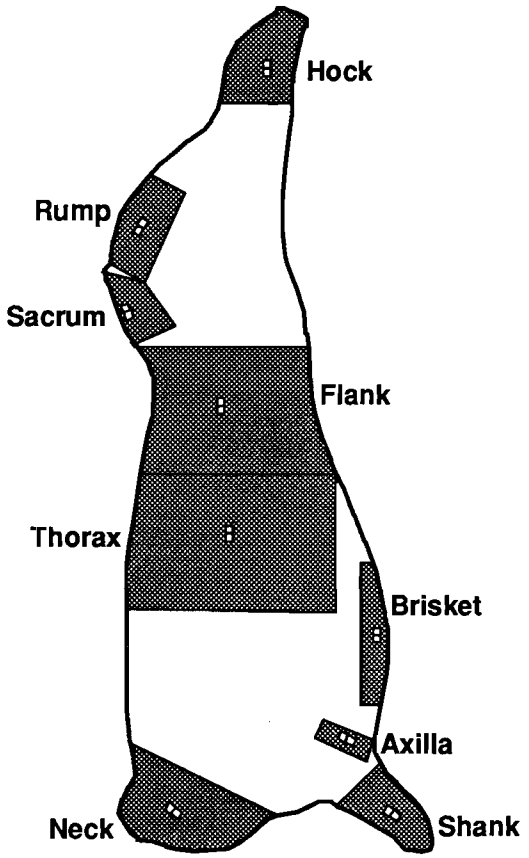

Figure 3. *Lateral aspect of beef carcass depicting the locations of the two adjacent sites from which equal sized (5 x 5 cm) samples were taken for microbiological analyses. Samples were taken at the center of cluster sites for which visual demerits were counted.*

Colony count variability and the relation with CANSIS demerits

Fifteen randomly selected carcasses from different lots were used to examine the between- and within-carcass variation of LMPN based on samples from the center of CS and relate LMPN to CANSIS demerits (not CANSIS-weighted) over the entire CS. Two adjacent samples were taken from 2-10 CS per carcass (total of 200 samples), and MPNGU/cm^2 was determined for each sample using 10 s stomacher time, TST plates, and 42 h incubation at 35°C for the HGMF. The four CANSIS demerits were also determined for the 100 carcass-CS combinations.

Estimates of the between- and within-carcass variation of LMPN were determined for each CS. An analysis of variance (*19*) was also carried out over the CS with variation due to carcasses and CS being included in the statistical model, and a cluster analysis (*17*) performed to group the CS for similarity of mean LMPN. Correlations were determined between LMPN for adjacent samples and between the mean LMPN for adjacent samples within a site and the total of the four CANSIS demerits (n = 100).

RESULTS

Enumeration of aerobic colonies

Effect of indicators on bacterial growth. TST plates (0.005%) supported growth of 16/17 genera and 23/24 strains of bacterial species tested. Only *Micrococcus luteus* failed to grow at this concentration of TTC. Even the gram-

positive species of the genera *Bacillus*, *Staphylococcus*, and *Streptococcus* (three species) grew well. All of the bacterial strains tested grew well on TS with Fast Green (0.025%).

The gram-positive species *Bacillus* spp., *Streptococcus* spp., and *Staphylococcus* spp. represented 75% of colonies (n = 60) grown from carcass surfaces on HGMF-TS with 0.005% TTC, but these same three species represented 85% of colonies (n = 69) when grown without TTC.

Effect of concentration of TTC on colony counts. There was no significant difference ($P > 0.05$) in the LMPN of colonies grown on TST plates at 0.01 and 0.005% TTC concentrations, but there was a significant difference between the 0.01 and 0.0025% TTC concentrations (geometric mean MPNGU/cm^2 of 59 ± 5 and 125 ± 10, respectively, $P < 0.01$). However, at a concentration of 0.0025% TTC and 42 h incubation, optical contrast was poor and overgrowth of colonies occurred beyond their hydrophobic boundaries. Contrast was optimal with 0.01% TTC, and this concentration was therefore considered as most appropriate for future studies.

Colony count repeatability. Wash times of 10 or 30 s for 32 samples yielded geometric mean MPNGU/cm^2 of 47 ± 3 and 79 ± 5, respectively, over 24 and 42 h incubations ($P < 0.01$). However, 30-s wash times were not practical because the resulting tissue-debris-rich suspensions produced excessive filtration times (4 min). Incubation times of 24 or 42 h (10-s wash time) of HGMF of the same sample yielded geometric mean MPNGU/cm^2 of 45 ± 2 and 82 ± 5, respectively ($P < 0.01$). There was no stomacher time x incubation time interaction effect ($P > 0.05$). Although TS plates with Fast Green tended to yield more MPNGU/cm^2 than TST plates (185 ± 42 versus 115 ± 20, respectively, $P > 0.05$), TS plates with Fast Green were not suitable because of overgrowth of colonies beyond hydrophobic boundaries as soon as 24 h of incubation.

Using samples from 10-s washes for which repeat HGMF and interpretations were obtained with TST plates, the relative variation attributed to samples, HGMF and interpretations was obtained for each of the 24- and 42-h incubation times (Table 1). The magnitude of the estimates of the variance components indicate the relative importance of these factors on the measurement of MPNGU/cm^2. About 98 and 94% of the variance of an individual LMPN estimate (repeatability) was due to sample to sample variation for incubation times of 24 and 42 h, respectively. Only 2% for 24 h incubation and 6% for 42 h incubation of the variation was due to interpretation and HGMF, respectively.

Localization of demerits in carcass regions. A significant study x region interaction ($P < 0.01$) was evident from the analysis of the subjectively assessed demerits per region which indicated that the magnitudes of differences among the regions was not consistent for studies 1 and 2. However, when regions with similar mean levels of the demerits were grouped into six clusters for each study (Fig. 2), the pattern of the clusters was similar. Also, differences between the regions were not always consistent between carcasses within a study.

Colony count variability and the relation with CANSIS demerits. MPNGU/cm^2 of the 200 samples taken from 10 CS of 15 carcasses ranged from 1-4781 and had a geometric mean of 170/cm^2. For the CS with the lowest counts (thorax) MPNGU/cm^2 ranged from 1-220 with a geometric mean of 22/cm^2, while for the site with the highest counts (axilla) the counts ranged from 4-3289/cm^2 with a geometric mean of 190/cm^2.

TABLE 1. *Analysis of variance and estimation of variance components for samples washed for 10 s and incubated for 24 or 42 h using TST plates.*

Source	df	MS+	VC+	%
24 h incubation				
Samples	53	1.7262	0.9496	97.9
HGMF	12	0.0205	0.0080	0.8
Interpretations	31	0.0125	0.0125	1.3
42 h incubation				
Samples	96	1.4168	0.8513	93.5
HGMF	58	0.0591	0.0591	6.5

+Mean square (MS) and variance component (VC) estimate in $\log_{10}$ units.

TABLE 2. *Estimates of between and within carcass variance components for MPNGU/cm^2 in CS thorax and shank.*

Site	Source	df	MS+	VC+	%
Thorax	Between	9	0.3488	0.0000	0.0
	Within	10	0.5091	0.5091	100.0
Shank	Between	9	1.4250	0.6288	79.0
	Within	10	0.1675	0.1675	21.0

+Mean square (MS) and variance component (VC) estimated in $\log_{10}$ units.

Although the number of carcasses and sites sampled were not large, it was evident that the variation in LMPN at the center of CS between and within carcasses was large. The within-carcass variance was generally smaller than the between-carcass component at a CS, but it was greater at some CS. The nature of the variation is indicated in Table 2 for the thorax and shank CS. These sites had the largest difference in the relative magnitudes of the between- and within-carcass variance components. Thus, not only can there be large differences in MPNGU/cm^2 of CS between carcasses but also within a CS. There was a significant effect of CS on LMPN ($P < 0.01$), and the CS were clustered into four groups with the following geometric means: neck (363 ± 161/cm^2); rump lateral and medial, hock and brisket (220 ± 98 to 281 ± 121/cm^2); rectum, sacrum, axilla, and flank (105 ± 47 to 162 ± 72/cm^2); and thorax (18 ± 8/cm^2). The low magnitude of the within-CS variation relative to the between-CS variation and the significant correlation of LMPN between adjacent samples ($r = 0.54$, $P < 0.01$, $N = 100$), indicated that there was similarity in the LMPN for adjacent samples within a CS. A significant but weak correlation ($r = 0.26$, $P < 0.01$, $n = 100$) between the mean of the LMPN for the adjacent samples at the center of CS and the total score of the four demerit types for the CS was evident.

DISCUSSION

This is the first report of the application of the HGMF interpreter system to samples excised from beef carcasses. Evaluation of chicken carcasses by this system has been reported as well as the counting of aerobic bacteria in various foods by manual or automated interpretations (*3-6,12*). Specifically, our recommended method counts MPNGU grown at 35°C for 42 h on TS agar with 0.01% TTC after a 10-s wash with a Colworth 80 Stomacher (see Results). Compromises were made in indicators (TTC over Fast Green), and wash time (10 over 30 s). The sensitivity of automated interpretation ranges from 1 to 5600 MPNGU/cm^2, and the maximum precision is at 670 MPNGU/cm^2 (95% confidence limits of 620-710 MPNGU/cm^2) (*18*). The method gives a repeatable count of colonies for the above conditions which is an unknown proportion of the total aerobic bacteria on the surface of the sample. The objective is to count as many bacteria as possible using a repeatable automated method. Total aerobic bacterial counts are not required because only relative contamination of groups of carcasses is at issue for hygiene assessment. Instead, each aerobic bacterium at any site is given the same high chance to be enumerated. More than 90% of the total variance in LMPN observations using the method is due to between-sample variation, which indicates that there is a high reliability in the between- and within-HGMF determinations of any given sample. Two workers can process 40 samples in 1.5 h (excluding time for sample collection) at a cost of $2.63 CAN/sample (1991), and one worker can interpret 40 HGMF in 1 h. The cost of the capital equipment was $18K CAN (1991).

The requirement for method repeatability excluded sampling by surface washing or swabbing of in situ samples. There are four reports of excision sampling for beef carcass surfaces (*1,7,9,20*), but none of these enumerated bacteria on groups of carcasses.

The objective of a study determines the method of sampling, sample size, and sample number. For example, washing of excised tissue samples of surfaces of beef carcasses yields at least 6 times more aerobic colonies on one side than swabbing by pad the same sized area at the same site on the other side of the same carcass (*1*). Similarly, the variability of the swab technique and/or area within the same carcass site on total viable counts was demonstrated by the recording of lower counts for large (100 cm^2) compared with smaller sized (10 cm^2) samples at the same carcass site. The proximity of these samples to each other was not stated (*10*). Swabbing is regarded as recovering only about 10-15% of bacteria on meat surfaces, and this depends on the method of swabbing and the type of surface sampled (*8*).

The magnitude of the variation in LMPN between the adjacent samples and weak, although significant correlation between counts for adjacent samples, suggests that more than one sample per site should be taken for carcass evaluation. Data from future carcass evaluations will be used to estimate the sample size required for group-carcass evaluations. In the only other study of adjacent samples, the means of microbial counts of excision samples (6.45 cm^2) at one site (neck) were found not to differ significantly. Correlation was not determined (*11*).

The variability observed in the MPNGU/cm^2 of two samples at the center of demerit clusters and its weak correlation (r = 0.26) to the demerit score of the same CS does not suggest that the CANSIS visual demerit evaluation of pre- or postwash carcasses be discontinued. To the contrary, at best the immediate visual evaluation should be complemented by the delayed microbiological evaluation of carcasses to monitor kill-floor hygiene.

Although there was only a very weak correlation between carcass demerit score and MPNGU/cm^2 at a CS (r = 0.26), for this level of carcass cleanliness, these sites were accepted for future carcass evaluations. The search for alternate sites with stronger correlations between demerits and MPNGU/cm^2 [possibly by total carcass evaluation (*20*)] was thought superfluous for the following reasons: a) the sites used are similar to those chosen by other workers including the most dirty sites for their conditions of carcass dressing (*14,15*); b) these sites are included by the inspectors and plant staff on the trim-line in detailed carcass inspection; and c) sites of carcass contamination are carcass dressing specific, and this distribution of sites is thought to best meet the needs of carcass evaluation for verification of HACCP systems at several plants.

The variance component estimates from this, and other studies in progress, will be used to determine the sample size required to estimate the average MPNGU at CS on the surface of a group of carcasses at one plant. In particular, determination of the number of samples per site, the number of sites per carcass, and the number of carcasses required is of interest.

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market classes may not have been detected because of the low prevalence of contamination. This conclusion is supported by the work of Spika et al. (*10*) who demonstrated a relationship between antemortem condemnation and *Salmonella* prevalence in pelvic fat samples from cows. That study involved fewer establishments and a higher prevalence of *Salmonella* contamination.

The carcass processing methods employed by high-volume establishments, although effective in reducing counts of microorganisms, had no measurable effect on the contamination of briskets or ground beef with *Salmonella* in this survey. *Salmonella* contamination may be more dependent on the animals brought to slaughter than on existing conditions in abattoirs. Previous studies (*5,9*) indicate that the prime source of *Salmonella* in slaughter establishments is the animals themselves.

The microbial contamination of cattle carcasses during slaughter is controllable to some extent by slaughter procedures. Some companies do so more effectively than others by the prevention of contamination during the dressing process and/or by the effective use of decontamination measures. More specific studies would be required to determine which measures were most effective in reducing total aerobic plate counts.

This study did not support the contention of Bryan et al. (*6*) that increased slaughter volume results in more finished product contamination by *Salmonella*. The prevalence of *Salmonella* contamination was found to be more closely associated with the health of animals brought to slaughter than on certain conditions in the slaughter establishments.

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